UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: February 15, 2011

SUBJECT: Copper Pyrithione: Hazard Assessment for Antifoulant Use

PC Code(s): 088001	DP Barcode(s)/No(s): D375749 and D369393
Decision No.: 373442	Registration No(s).: 1258-RGEE – Copper Omadine Powder AF
Petition No(s).: NA	Regulatory Action: Hazard Assessment
Risk Assess Type: Single Chemical	Case No(s).: NA
TXR No.: 1,003,204	CAS No(s): 14915-37-8
MRID No(s).: See reference list on pages 23 and 24.	40 CFR: None

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Attached is RASSB's hazard assessment of copper pyrithione, proposed for use as an active ingredient in antifoulant paint.

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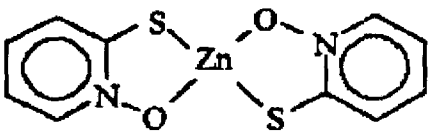
0.0 BACKGROUND

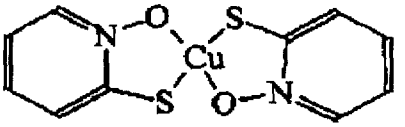
The registrant, Arch Chemical (Arch) has submitted an application for registration of a manufacturing use product containing copper pyrithione (copper omadine, CuPT), as the sole new active ingredient, for use only in formulating antifoulant paints. This application for registration was originally submitted in 2002. Arch requests that sodium pyrithione (sodium omadine, NaPT) and zinc pyrithione (zinc omadine, ZnPT) data be used as surrogate or bridging data for the copper pyrithione registration.

The chemical structures of these pyrithiones are listed below:

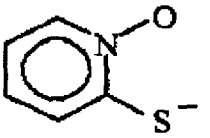
Structures:



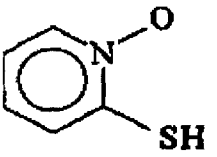
Zinc pyrithione



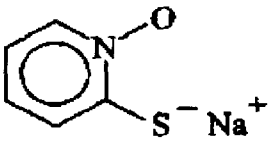
Copper pyrithione



Ionized pyrithione



Unionized pyrithione



Sodium pyrithione

Toxicology data originally submitted for CuPT included acute oral, dermal, and inhalation toxicity studies, skin and eye irritation studies, a skin sensitization study, 28-day repeated oral toxicity dosing studies conducted in the rat and monkey, an *in vitro* bacterial gene mutation study, and *in vitro* cytogenetic studies.

On May 26, 2004, the submitted information was discussed by the Antimicrobials Division Toxicology Endpoint Selection Committee (ATDC). The ADTC noticed that CuPT has lower water solubility compared with sodium pyrithione and zinc pyrithione. The 2004 ADTC concluded that insufficient data were available for bridging between copper and zinc pyrithione. The primary reasons are:

Different physical chemical properties of copper, sodium, and zinc pyrithione will affect the absorption/bioavailability of the chemical;

1. A similar metabolic profile between pyrithiones does not necessarily relate to the mode of action for causing effects of concern;
2. Because emesis was observed in both sodium and zinc pyrithione in the subchronic monkey study, it is difficult to estimate the real exposed concentration; and
3. Signs of neurotoxic effects (skeletal muscle wasting, hind leg paralysis) were noticed in the 90-day rat oral studies for both sodium pyrithione and zinc pyrithione. There was no 90-day copper pyrithione study to compare results with.

To address the ADTC's concerns, Arch conducted a 90-day oral neurotoxicity study (MRID 47023701), and comparison metabolism studies between copper pyrithione and zinc pyrithione (MRID 47844501). After reviewing the information, RASSB concluded that cooper pyrithione and zinc pyrithione data cannot be bridged (Chen, 2008). Reasons are listed below:

1. Different physical chemical properties of copper, sodium, and zinc pyrithione will affect the absorption/bioavailability of the chemical

In comparing the metabolism study results for copper pyrithione (MRID 4784451) and zinc pyrithione (MRID 47751201), the time to maximum concentration for pyrithione (PT) derived from CuPT is approximately three times longer than when derived from ZnPT, and Cmax values from CuPT are less than half those from ZnPT. The 0−24 h Area under the plasma concentration time curve (AUC) for PT derived from CuPT is nearly twice that for PT derived from ZPT suggesting that absorption of PT derived from CuPT is much more prolonged than from ZnPT. This prolonged absorption likely produces the longer apparent half-life of PT derived from CuPT than from ZnPT as the half-life of PT should be identical regardless of its source. **Therefore, although CuPT and ZnPT may have similar biological metabolite(s) in the body, the toxicokinetics may be different.**

2. Different mode of action may trigger copper pyrithione related toxicity through inhalation exposure route.

Copper pyrithione is much less water soluble when compared with both zinc pyrithione and/or sodium pyrithione. At pH 7.0, at 20°C, zinc pyrithione solubility in water (6ppm) is 100 times more than copper pyrithione (0,06ppm). RASSB concluded similar kind of concern may occur at lower exposure concentration through inhalation route. The mode of action triggering the toxic effects through the inhalation route for CuPT may be completely different from the mode of action causing systemic effects and/or ZnPT-caused inhalation toxic effects

3. Sex differences in sensitivity to the toxic effects from copper pyrithione exposure .

Female rats are more sensitive to copper pyrithione exposure through the oral route when compared to male rats. Some of the toxic effects may not be reversible after exposure is stopped. There is no developmental toxicity study associated with copper pyrithione exposure. Therefore, Agency cannot compare the potential developmental effects associated with copper pyrithione exposure.

Therefore, RASSB decided that one 90-day inhalation in rat and one prenatal developmental in rat are needed. For dermal exposure assessments, a conservative assumption of 100% dermal absorption will be used for dermal exposure risk assessment.

In 2009, instead of conducting the data agency requested, Arch continued to pursue the possibility of bridging the data between copper and zinc pyrithiones. Arch submitted one set of new acute studies (acute studies through oral, dermal and inhalation routes and one dermal sensitization study), and five additional studies:

1. A 4-Week CuPT inhalation toxicity MRID 48006403;

2. A 4-Week ZnPT inhalation toxicity MRID 48006404;

3. An inhalation of ^{14}C-Copper Pyrithione metabolism study (MRID 48006401);

4. An inhalation of ^{14}C-Zinc Pyrithione metabolism study (MRID 48006402); and

5. One rat Copper Pyrithione developmental study.

Based on the additional submitted data, the ADTC met on 11/04/2010 to discuss the significance of the new findings. The ADTC determined that:

(1) With the newly available 28-day inhalation study conducted with CuPT and the inhalation metabolism study, the available information was adequate to address the issues associated with inhalation hazard concern. Therefore, the 90-day CuPT inhalation study requirement can be waived;

(2) Based on the newly submitted inhalation metabolism studies, the ADTC agreed that the high death rate in the CuPT acute inhalation toxicity study may be caused by crystal (dust) formation due to its low water solubility as the registrant suggested;

(3) The mode of action for the hindlimb weakness caused by pyrithiones (NaPT, ZnPT and CuPT) is still not clearly defined;

(4) The available study results between ZnPT and CuPT show similar hazard end-points. The ADTC agreed that CuPT and ZnPT data can be bridged up to intermediate-duration exposure scenarios (0-6 month exposure), including developmental effects.

(5) The different physical/chemical properties between CuPT and ZnPT result in varying absorption and bioavailability between the two chemicals. This is demonstrated by the oral metabolism study for CuPT, where a longer biological half life is observed with CuPT when compared with ZnPT. With long-term exposure scenarios, the ADTC is concerned that the slower absorption and excretion may result in differences in body burden, especially in chronic exposure conditions.

(6) There are no reproductive toxicity data or other long-term studies (chronic and/or cancer studies) to compare CuPT and ZnPT. Therefore, the ADTC does not consider it

appropriate to bridge the databases between CuPT and ZnPT for reproductive effects and chronic use conditions.

(7) Since the antifoulant paint use is the only currently intended use for CuPT and no chronic exposure is expected, the databases for CuPT, ZnPT and NaPT can be bridged for this risk assessment.

The hazard Assessment associated with CuPT for antifoulant use is prepared based on the ADTC conclusions.

1.0 HAZARD CHARACTERIZATION

In comparing the toxicology data and metabolism/disposition studies (both oral and inhalation routes), it was agreed that copper pyrithione (copper omadine, CuPT) and zinc pyrithione (zinc omadine, ZnPT) data can be bridged up to intermediate-duration exposure scenarios (0-6 month exposure), including developmental effects. There are no reproductive toxicity data or other long-term studies (chronic and/or cancer studies) to compare CuPT and ZnPT. Therefore, it is not appropriate to bridge the databases between CuPT and ZnPT for reproductive effects and chronic use conditions.

CuPT is corrosive to eyes and mild or slight irritating to skin. CuPT is not a dermal sensitizer. It is of moderate acute toxicity by oral, dermal, or inhalation routes of exposure.
As indicated by the 9-day rat toxicology study, 28-day rat oral study, 90-day rat oral neurotoxicological study and rat developmental study, hind limb weakness were consider as the primary effects of concern. It appears female is more sensitive to the effect.

In the inhalation study, test substance-related mortality was observed in the 0.005 mg/L group females. At 0.0015 mg/L group decreased food consumption in the females, effects on BALF parameters (increased LDH, total protein, total cell counts, lymphocytes, alveolar macrophages, and neutrophils) and histopathology in the lungs (subacute inflammation and alveolar macrophages) in both sexes were noticed.

There is one developmental study associated with oral exposure to CuPT. At a dose of 10 mg/kg/day in this study , decreased body weight, body weight gain, and food consumption in both sexes was observed as well as mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. No developmental effects were noticed in any dose tested.

There is no reproductive study for CuPT. No chronic toxicity or carcinogenicity studies are available to assess the chronic toxicity or carcinogenicity of CuPT. There is a battery of negative mutagenicity studies.

2.0 AVAILABLE DATABASE

The toxicology studies that have been submitted for CuPT are shown in **Table 1**.

Table 1. Toxicology Database for Copper Pyrithione

Guideline Number	Test	Technical		
		Required	Satisfied	MRID Number
OPPTS 870.3050	28-day Oral Study - Rat	N	Y	45774311
OPPTS 870.3050	28-day Oral Study - Monkey	N	Y	45774312
OPPTS 870.3465	28-day – inhalation Study	Y	Y	48006403
OPPTS 870.3700	Developmental Toxicity - Rat	Y	Y	48158006
OPPTS 870.5375	A Chromosomal Aberration - in Cultured Chinese Hamster Cells	Y	Y	45774313
OPPTS 870.6200	90-day Oral Neurotoxicity Study - Rat	Y	Y	47023701
OPPTS 870.7485	Oral metabolism - Rat	N	N	45774326
OPPTS 870.7485	Oral Metabolism – Rat	Y	Y	47844501
OPPTS 870.7485	Inhalation metabolism	N	Y	48006401
Non-Guideline	9-Day Toxicity and Metabolism Study in Rats	N	Y	45774326

Y - Yes; **N** - no

In this toxicology assessment chapter, the toxicology database of copper pyrithione is evaluated, and the toxicological endpoints used for risk assessment are selected. In addition, the data gaps in the database are also identified.

3.0 HAZARD ASSESSMENT

3.1 Acute Toxicity

The acute toxicity data on CuPT is summarized below in Table 2. CuPT is corrosive to eyes and mild or slight irritating to skin. CuPT is not a dermal sensitizer. CuPT is of moderate acute toxicity by oral, dermal, or inhalation routes of exposure.

Table 2. Acute toxicity decisions for Copper Pyrithione

Guideline No.	Study Type	MRID number (s)	Results	Toxicity Category
OPPTS 870.1100 [§ 81-1]	Acute Oral	45774305 and 4815002	LD50 = 1075 mg/kg (M); 500-1000 mg/kg (F) LD50 > 200 mg/kg	II
OPPTS 870.1200 [§81-2]	Acute Dermal	48158003	LD50 > 400 mg/kg 2 rats dead at 2000 mg/kg	II
OPPTS 870.1300 [§ 81-3]	Acute Inhalation	48158004	LC_{50} (M) 0.14 (0.11-0.18) mg/L (F) 0.17 (0.13-0.23) mg/L (C) 0.15 (0.13-0.18) mg/L	II
81-4	Primary Eye Irritation	45774307	Corrosive	
81-5	Primary Skin Irritation	45774308	Mild or slight	
OPPTS 870.2600 [§ 81-6]	Dermal Sensitization	41580011	Non-sensitizer	

3.2 Short-term Oral Toxicity

There is one short-term (9-Day) toxicity and metabolism study in rats (MRID 45774326). In the study, female Sprague-Dawley rats were treated with CUPT in aqueous 0.5% Darvan (sodium polynaphthalenesulfonate) by daily oral gavage in a dose volume of 5 mL/kg. An initial range-finding study was performed to investigate the toxicity. In this study, animals (3/dose group) were dosed for 9 consecutive days with vehicle or 4, 6, 9, or 12 mg/kg/day CuPT. Mortality, clinical signs, body weight, muscle mass, and muscle tone were reported. The results from this study were used to select the doses for a metabolism study; the doses selected represented toxicologically equivalent doses. In the metabolism study, animals (5/dose group) were treated with 4 mg/kg/day CuPT; radiolabeled test compounds were administered on Days 7 and 8. Mortality, clinical signs, body weight, food consumption, excretion profile, blood partitioning, muscle mass, muscle tone, and metabolite characterization were reported.

In the range-finding study, CuPT treatment of rats resulted in neurological signs such as irregular gait, lethargy, and paralysis. CuPT treatment resulted in a dose-dependent decrease in body weight. In the CuPT-treated animals, muscle mass and tone were greatly reduced.

Dose of 4 mg/kg/day CuPT was chosen for the metabolism study, as these doses provided the most similar toxicological effects, based on clinical signs. All animals were slightly lethargic on Day 5 at 4 hours post-dosing. At 0.5 hours post-dosing, the following findings were observed: slightly lethargic, non-responsive, prostrate, salivating and lachrymating. These signs were first observed at Days 5 or 6. The animals maintained their initial body weight through Day 6. After administration of the radiolabeled compound (Days 7 and 8), losses of 31 g the CuPT treated groups, respectively. In the CuPT-treated animals, muscle mass was slightly reduced in 1 animal and greatly reduced in 3 animals; likewise, no muscle tone was noted in 3 rats and 1 rat only had moderate tone.

This study is classified as acceptable/non-guideline.

3.3 Subchronic Oral Toxicity Studies

There are three subchronic oral studies (one 28-day Cynomolgus monkey oral study, one 28-day rat feeding study, and one 90-day rat feeding study) available in the toxicology database.

Rat 28-day oral Study (MRID 45774311)

In a repeat dose toxicity study (MRID 45774311), copper pyrithione (100 % purity) by gavage at doses of 0, 0.6, 2.5 or 10 mg/kg/day to 5 or 10 male and females Crj:CD (SD) rats/group for 28 days. Further groups of control and high dose animals (5/sex) were retained for an additional 14 days to assess recovery from any adverse effects. Due to the deaths occurring in high dose females, this recovery group consisted of only 2 animals. The vehicle employed was 0.5 % carboxymethylcellulose (sodium salt).

At 10 mg/kg/day, two females were sacrificed *in extremis* on Day 17 of the dosing period. Additionally, one 10 mg/kg/day female was found dead on Day 2 of the recovery period. These three animals exhibited the following clinical signs prior to death or euthanasia: emaciation; decreased spontaneous activity, piloerection, ataxic gait, paralysis of the hind leg, urine-stained abdomen, reddish eye gum; prone position; and lateral position. Additionally, hypothermia and bradypnea were noted in the females sacrificed in moribund condition, and a trace of reddish rhinorrhea was observed in the animal found dead. Among the surviving 10 mg/kg/day females, similar clinical signs of emaciation, piloerection, decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg; reddish eye gum; urine-stained abdomen; and prone position were found. However, almost all of these findings decreased in severity or occurrence during the final week of dosing. During the initial recovery period, decreased spontaneous activity, ataxic gait, and piloerection were still observed in 1 or 2 females, but these abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females during the recovery period, but these animals appeared to be recovering.

No treatment-related clinical signs were noted in the 10 mg/kg/day males, other than slight emaciation in three males on Days 21 to 28 or on Days 0-2 of the recovery period,

Body weights were decreased ($p<0.05$) by 10-13% in the 10 mg/kg/day males during Weeks 3 and 4 and by 19-36% in the females during Weeks 2-4. During the recovery period, body weights remained decreased by 13% in these males during Week 1 and by 25-33% in the females during Weeks 1 and 2. Body weight gains were decreased ($p<0.05$) by 18-39% in the males during Weeks 2-4. The females at this dose actually lost significant ($p<0.01$) weight during Week 2 (-0.6 g) and Week 3 (-18.8 g) compared to gains of 24.6 and 28.6 g, respectively, in the controls. Overall (Weeks 0-4) body weight gain was decreased by 22% in the males and 76% in the females. During the recovery period, body weight gains were increased compared to controls by 28% in the males during Week 2 and by 82-123% in females during Weeks 1 and 2. Food consumption was decreased ($p<0.01$) by 11-12% in the males during Weeks 3 and 4 and by 34-37% in the females during Weeks 2 and 3. Food consumption was similar to controls in both sexes during the recovery period.

At the high-dose, treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period. Microscopic observations of muscle fiber atrophy were observed in the gastrocnemius, soleus, flexor digitorum longus, and anterior tibial muscles in all (5/5) females at this dose compared to 0/5 controls. Additionally, muscle fiber atrophy of the biceps femoris was found in 2/5 females at 10 mg/kg/day compared to 0/5 controls. In the high-dose males, muscle fiber atrophy in the anterior tibial muscle was observed in 2/5 rats compared to 0/5 controls. The severity of the muscle fiber atrophy was generally very slight to slight.

No treatment-related effects were observed at 0.6 or 2.5 mg/kg/day in either sex.

The LOAEL was 10 mg/kg/day based on decreased body weight, body weight gain, and food consumption in both sexes and on mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. The NOAEL is 2.5 mg/kg/day.

Cynomolgus Monkey 28-day oral Study

In a repeat dose toxicity study, groups of cynomolgus monkeys (MRID 45774312), copper pyrithione (100 % purity) were administered to groups of Cynomolgus monkey (6/sex) via gelatine capsules at doses of 0, 21 11, 22 or 44 mg/kg/day, for 28 days. Two animals from the control and high dose groups were retained for an additional 14 days to assess recovery from any adverse effect. The following examinations were conducted at the end of the recovery and treatment phases; ophthalmoscopy, urinalysis, haematology, and gross necropsy and histopathology, including of the sciatic nerve and quadriceps muscle. No deaths occurred. Clinical signs of toxicity were confined to diarrhoea and green stools at the high dose and sporadic instances of loss of appetite at 11 mg/kg/day, becoming widespread at 22 mg/kg/day. No body weight, ophthalmoscopy, or urinalysis changes were observed. Haematological changes were confined to high dose males at the end of the test phase only: statistically significant decreases in erythrocyte numbers (by 22 %), haematocrit (by 18 %) and haemoglobin concentration (by 19 %) and increased platelets (by 45 %) were observed. All these changes were comparable to the

controls at the end of the recovery period. Following clinical chemistry analysis, a statically significant increase (330 and 400 % in males and females, respectively) in serum triglycerides was observed at 44 mg/kg/day. These increases were apparent from week 1 of the study, but were comparable to controls at the end of the recovery period. The only treatment-related changes observed at necropsy were statistically significant increases (by approximately 40 and 50 % in both sexes) in absolute and relative liver weight respectively at 44 mg/kg/day, at the end of the test period. However, no treatment-related changes were observed at the end of the recovery period. No histopathological abnormalities were observed in either period. Overall, a NOAEL was not identified; as loss of appetite was observed at all dose levels. **A LOAEL can be identified of 11 mg/kg/day, on the basis of findings from this study.**

90-Day Rat Oral Neurotoxicity Study

In a subchronic neurotoxicity study (MRID 47023701), copper pyrithione (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was administered orally via gavage (10 mL/kg) once daily to 16 Sprague-Dawley rats/sex/dose at doses of 0 and 2.25 mg/kg/day and to 10 rats/sex/dose at 0.5 and 1.25 mg/kg/day for 91 consecutive days. At the end of the dosing period, a subset of 10 rats/sex/dose from the control and 2.25 mg/kg/day groups were allowed to recover for an additional 6 weeks. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals at pre-dosing and Weeks 2, 4, 8, and 13. At study termination, 5 rats/sex/group were anesthetized and perfused *in situ* for neuropathological examination.

The tissues from the perfused animals in the control and 2.25 mg/kg/day groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Acceptable positive control data were provided. No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. One female (#16197) was sacrificed *in extremis* on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material in the range finding study. An increased ($p \leq 0.01$) incidence of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females. During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of Compound Motor Action Potential (CMAP) (decr 28%) that remained reduced, but to a lesser extent (decr 13%), following the 6-week recovery period. Treatment-related neuropathological were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis.
At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in 4/10 males and 3/10 females.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

3.4 Subchronic Inhalation Toxicity

There is one 28-day inhalation study associated with copper omadine exposure (MRID 48006403). In the study Copper Omadine® (97% a.i., Lot no. 0103239911) was administered as a dust aerosol to 15 Crl:CD(SD) rats/sex/concentration by nose-only exposure at concentrations of 0 (air), 0.5, 1.5, or 5.0 mg/m^3 (equivalent to analytical concentrations of 0, 0.0005, 0.0015, and 0.0049 mg/L) for 6 hours per day, 5 days/week for up to 4 weeks (up to 20 exposure days). Five rats/sex/concentration were euthanized following 1, 2, and 4 weeks of exposure and subjected to a gross necropsy. Selected organs were weighed and examined microscopically. A special emphasis was placed on the evaluation of pulmonary effects, including assessment of bronchoalveolar lavage fluid (BALF) parameters and microscopic examination of the lungs.

Test substance-related mortality was observed in the 0.005 mg/L group females. Two females (nos. 6047 and 6062) were found dead on Day 12, and one female (no. 6029) was found dead on Day 19. Test substance-related clinical observations noted for the animals found dead included hypoactivity, thin body condition, and body cool to touch. Additionally, female no. 6029 was noted with decreased defecation on Day 17, as well as dermal atonia and impaired muscle coordination on Day 18. The majority of these clinical observations were noted within 24 hours of death. However, the cause of death for these three females was undetermined. Microscopic findings in the lungs (broncho-interstitial pneumonitis characterized by subacute inflammation and an increase in alveolar macrophages) and skeletal muscle (atrophy and degeneration) were similar in nature and severity to that which occurred in females at the scheduled necropsy on Day 26. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26. Test substance-related clinical observations were noted for the 0.005 mg/L group females surviving to scheduled termination. These findings included the following: thin body condition noted by Day 11 and continuing throughout the duration of the study, dermal atonia noted from Days 12 to 19, and pale extremities noted in a single animal on Day 20. Impaired use of right and left hindlimbs was noted for two animals ranging from Day 20 to 24. Other treatment-related clinical findings in this group were limited to yellow and/or red material on various parts of the body (including ocular, nasal, urogenital, and anal).

Treatment-related effects on body weights were observed in both sexes at 0.005 mg/L. In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased on Day 4 and continued to be decreased (not significant) throughout the remainder of the study. A significant ($p \leq 0.01$) body weight loss was noted for Days 0-4 in the 0.005 mg/L males (-10 g) compared to a gain of 11 g in the controls, and cumulative body weight gains were 42-56% lower than controls for all other intervals throughout the study; these decreases were statistically significant for all intervals, except for Days 0-25. In the 0.005 mg/L females, body weights were significantly ($p \leq 0.01$) decreased throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 90% lower ($p \leq 0.01$) than controls for Days 0-4, and cumulative body weight losses of 22-48 g were noted for each of the remaining intervals throughout the study in this group compared to

body weight gains of 23-48 g in the control group. Food consumption was decreased ($p \leq 0.05$) in the 0.0015 mg/L females for Days 0-4. Additionally at 0.005 mg/L, food consumption was decreased ($p \leq 0.01$) for Days 0-4 in the males and for Days 0-4, 4-11, and 11-18 in the females.

Treatment-related effects on BALF samples were found at 0.0015 and 0.005 mg/L in both sexes. Lactate dehydrogenase was increased over controls at 0.0015 mg/L in the males on Days 12 and 26 and in the females on Day 5 and at 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total protein levels were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total cell counts and the number of lymphocytes were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Day 5. Alveolar macrophages were increased in the 0.0015 and 0.005 mg/L males throughout the study and in the 0.005 mg/L females on Day 5. With the exception of the 0.005 mg/L females on Day 26, the number of neutrophils was increased throughout the study in the 0.0015 and 0.005 mg/L males and females.

Examination of the BALF leukocyte differential data showed that the proportion of neutrophils increased with concentration and time in both sexes at 0.0015 and 0.005 mg/L, with the exception of the 0.005 mg/L females on Day 26. The remaining three females exposed to 0.005 mg/L had apparently recovered (following the final exposure on Day 18), and neutrophils were not present in the BALF on Day 26. The proportion of lymphocytes was higher than controls in the 0.005 mg/L males and females on Days 5 and 12. Due to these increases in neutrophils and lymphocytes, the proportion of alveolar macrophages was lower in the 0.005 mg/L males on Days 5, 12, and 26 and females on Days 5 and 12. The proportion of alveolar macrophages was slightly lower than controls at 0.0015 mg/L in both sexes at all three intervals.
At 0.0015 mg/L, relative (to body weight) lung weights were increased ($p \leq 0.01$) over controls in the males on Day 12. The following increases ($p \leq 0.05$) in lung weights were observed in the 0.005 mg/L animals: (i) relative to body weight and relative to brain weight in the males and females at Day 5; (ii) absolute and relative to body weight in the males at Days 12 and 26; and (iii) relative to body weight in the females at Days 12 and 26. Terminal body weights were decreased ($p \leq 0.05$) at this concentration in the males on Day 5 and in the females on Days 12 and 26. Subacute inflammation was observed in the lungs in males at 0.0015 mg/L (4/15) and 0.005 mg/L (13/15) compared to controls (0/15) and in the females at 0.005 mg/L (9/12) compared to controls (1/15). The severity of subacute inflammation of the lungs ranged from minimal to moderate in the males and from minimal to mild in the females. Minimal alveolar macrophages were observed in the 0.0015 mg/L males (5/15) and females (3/15), and mild alveolar macrophages were found at 0.005 mg/L in the males (12/15) and females (9/12) compared to controls (0/15 males; 1/15 females). Perivasculitis was found in the 0.005 mg/L males (1/15) and females (2/12) compared to controls (0/15 males; 1/15 females). These findings were considered to be due to direct effects of the test material on the respiratory tract. With the exception of the perivasculitis in the females, the findings increased in severity with increasing concentration.

Enlarged bronchial lymph nodes were noted in one 0.005 mg/L female on Day 5, one 0.0015 mg/L male on Day 12, two 0.005 mg/L males on Day 12, one 0.005 mg/L female on Day 12, and one 0.005 mg/L male on Day 26. Enlarged mediastinal lymph nodes were noted in one 0.0015 mg/L male on Day 12, one 0.005 mg/L male on Day 12, two 0.005 mg/L females on Day 12, and

one 0.005 mg/L male on Day 26. These findings, noted at gross necropsy, corresponded to lymphoid hyperplasia confirmed upon microscopic examination.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to moderate degeneration (4/15 treated vs 0/15 controls) and mild to moderate atrophy (5/15 treated vs 0/15 controls).

The LOAEL is 0.0015 mg/L based on decreased food consumption in the females, effects on BALF parameters (increased LDH, total protein, total cell counts, lymphocytes, alveolar macrophages, and neutrophils) and histopathology in the lungs (subacute inflammation and alveolar macrophages) in both sexes. The NOAEL is 0.0005 mg/L.

This 28-day study is classified as **acceptable/non-guideline.**

3.5 Chronic Toxicity and Carcinogenicity Studies.

No chronic toxicity or carcinogenicity studies are available to assess the chronic toxicity or carcinogenicity of CuPT.

3.6 Developmental Toxicity

In a rat developmental toxicity study (MRID 48158006), copper pyrithione (99.6%; batch/lot #990128) in 1% carboxymethyl cellulose was administered via daily oral gavage in a dose volume of 10 mL/kg to 25 time-mated Sprague-Dawley rats/dose group at doses of 0, 0.5, 1.5, or 3 mg/kg/day from gestation days (GD) 6-15. On GD 20, all dams were euthanized; each dam's uterus was removed via cesarean section and its contents examined. Fetuses were examined for external, visceral, and skeletal malformations and variations.

In the 3 mg/kg/day group, body weight gains (relative to GD 6, start of dosing) were decreased by 6-31% throughout the dosing period and food consumption was decreased ($p<0.01$) by 10% during GD 6-9. **The maternal LOAEL is 3 mg/kg/day, based on decreases in body weight gain and food consumption during the dosing period. The maternal NOAEL is 1.5 mg/kg/day.**

There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses and no effects of treatment on the numbers of litters, live fetuses, or total resorptions. Similarly, fetal weights, sex ratio, and pre- and post-implantation losses were unaffected by treatment. There were no treatment-related external, visceral, or skeletal variations or malformations. **The developmental LOAEL was not observed. The developmental NOAEL is 3 mg/kg/day.**

Based on the available study result, ZnPT and CuPT show similar hazard end-points. The ADTC agreed that CuPT and ZnPT data can be bridged up to intermediate-duration exposure scenarios (0-6 month exposure), including developmental effects. Therefore one ZnPT dermal developmental toxicity study (MRID 46534001) was also considered. In the study, zinc pyrithione (98.3% a.i., batch/lot # 0108244691) was dermally administered to 23-25 Crl:CD® (SD)IGS BR VAF/Plus® rats/dose at dose levels of 0, 10, 15, 30, or 60 mg/kg bw/day from day 0 through 20 of gestation.

Dermal administration of zinc pyrithione resulted in maternal toxicity at 30 and 60 mg/kg/day. Treatment-related effects at 60 mg/kg/day included clinical signs of toxicity (grade 1 erythema; grade 1 flaking; limited or no use of hindlimbs; shuffling gait; dehydration; low carriage; chromodacyorrhea; emaciation; chromorhinorrhea; hunched posture; decreased body weight and body weight gain, decreased uterine weight, decreased corrected body weight and corrected body weight gain, decreased absolute and relative feed consumption, and decreased muscle tone and mass.

At 30 mg/kg/day, there was an increase in the number of dams with limited use of hindlimbs, shuffling gait, decreased body weight and body weight gain, decreased corrected body weight and corrected body weight gain, and decreased absolute feed consumption. There were no treatment-related maternal effects at 10 or 15 mg/kg/day. **The maternal LOAEL is 30 mg/kg bw/day, based on an increase in the number of dams with limited use of hindlimbs, shuffling gait, decreased body weight and body weight gain, decreased corrected body weight and corrected body weight gain, and decreased absolute feed consumption. The maternal NOAEL is 15 mg/kg bw/day.**

Developmental toxicity occurred at 60 mg/kg/day. Treatment-related findings at 60 mg/kg/day included decreased fetal weight (total, male, and female), an increase in the percentage of litters and fetuses with incomplete ossification of the sternal centra, an increase in the percentage of fetuses with wavy ribs, and a decrease in the ossification site averages for the caudal vertebrae, forelimb phalanges and metacarpals, and hindlimb phalanges and metatarsals per fetus per litter. There were no treatment-related developmental effects at 10, 15, or 30 mg/kg/day.

The developmental LOAEL is 60 mg/kg bw/day, based on decreased fetal weight (total, male, and female), an increase in the percentage of litters and fetuses with incomplete ossification of the sternal centra, an increase in the percentage of fetuses with wavy ribs, and a decrease in the ossification site averages for the caudal vertebrae, forelimb phalanges and metacarpals, and hindlimb phalanges and metatarsals per fetus per litter. The developmental NOAEL is 30 mg/kg bw/day.

3.7 Reproductive toxicity

No reproductive toxicity studies are available for CuPT.

3.8 Neurotoxicity

It is understood through the available rat toxicology studies that hindlimb weakness is considered a primary effect of concern for all the pyrithiones. It appears there is a sex-related sensitivity to the toxic effects from copper omadine exposure (female being more sensitive to the effects). In the 90-day neurotoxicity study (MRID 47023701), neuropathological examinations were conducted and the findings were limited to the skeletal muscle sections, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis. Similar findings were noticed in the 9-day toxicity study MRID 45774326), 28-day rat oral study (MRID 45774311), rat developmental study (MRID 48158006), and other pyrithiones (CuPT and ZnPT) studies.

3.7 Mutagenicity and Carcinogenicity

There is a battery of negative mutagenicity studies.

Salmonella typhimurium Assay/ CHO-HGPRT Assay

In MRID 45785505, copper pyrithione was examined for mutagenicity in Salmonella strains TA98, TA100, TA1535, and TA1537 and in one strain of E. coli (WP2uvr A) in the presence and absence of metabolic activation. No increase in the number of revertant colonies was observed in this study for any of the strains tested.

In MRID 45804101, copper pyrithione was tested using the CHO/HGPRT assay in the presence and absence of metabolic activation. In the assay, no cultures with mutant frequencies were observed over solvent-treated controls.

The results of these studies appear to show that, similar to ZnPT and NaPT, there is no evidence of a mutagenic effect associated with CuPT in either the absence or presence of metabolic activation.

Chromosome Aberration Test in Monkey Lymphocytes

This study (MRID 45774314) was an in vitro chromosomal aberration test using peripheral lymphocytes obtained from cynomolgus monkeys. This test used both the direct method test and the metabolic activation test. As noted in the report, "In the direct method test, no significant difference was noted in the average frequencies of structural aberrations or polyploidy between the test article treated groups and the control group.

In the metabolic activation method test, no significant difference was noted in the average frequencies of structural aberrations or polyploidy between the results of the metabolic activation method test with S9 Mix and the results of the metabolic activation method test without S9 Mix. Further, in the metabolic activation method test, no significant difference was noted in the avenge frequencies of structural aberrations or polyploidy between the test article treated groups and the control group.

Therefore, under these two test conditions, the test results indicate that CuPT did not induce clastogenicity in the cynomolgus monkey peripheral lymphocyte. "

Chromosomal Aberration Test in Chinese Hamster Ovary Cells

In this study, clastogenic potential of CuPT was evaluated in Chinese Hamster Ovary cells (CHL/IU cell line). As noted in the report," In the direct method test, no significant difference was noted in the average frequencies of structural aberrations or polyploidy between the test article treated groups and the control group.

In the metabolic activation method test, no significant difference was noted in the average frequencies of structural aberrations or polyploidy between the results of the metabolic activation method test with S9 Mix and the results of the metabolic activation method test without S9 Mix. Further, in the metabolic activation method test, no significant difference was noted in the average frequencies of structural aberrations or polyploidy between the test article treated groups and the control group."

4.0 HAZARD ENDPOINT SELECTION

The doses and endpoints for short-term and intermediate-term occupational or residential exposure have been selected for CuPT for antifoulant paint use. **Table 3** summarizes the toxicological dose and endpoints for CuPT for use in human risk assessments.

4.1 ACUTE DIETARY (Acute Reference Dose, aRfD)

An acute dietary endpoint (acute Reference Dose) was not selected for CuPT because the chemical has no food uses and dietary exposure is not expected.

4.2 CHRONIC DIETARY (Chronic Reference Dose, cRfD)

A chronic dietary endpoint (chronic Reference Dose) was not selected for CuPT because the chemical has no food uses and dietary exposure is not expected.

4.3 OCCUPATIONAL / RESIDENTIAL EXPOSURE

4.3.1 Dermal Absorption

A dermal absorption factor for CuPT is not required since a dermal NOAEL is available from a study conducted with ZnPT.

4.3.2 Incidental Oral - (Short- and Intermediate-term)

Study Selected: 90 Day Oral Neurotoxicity Study Rat

MRID No.: 47023701

Executive Summary: In a subchronic neurotoxicity study (MRID 47023701), copper omadine (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was administered orally via gavage (10 mL/kg) once daily to 16 Sprague-Dawley rats/sex/dose at doses of 0 and 2.25 mg/kg/day and to 10 rats/sex/dose at 0.5 and 1.25 mg/kg/day for 91 consecutive days. At the end of the dosing period, a subset of 10 rats/sex/dose from the control and 2.25 mg/kg/day groups were allowed to recover for an additional 6 weeks. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals at pre-dosing and Weeks 2, 4, 8, and 13. At study termination, 5 rats/sex/group were anesthetized and perfused in situ for neuropathological examination. The tissues from the perfused animals in the control and 2.25 mg/kg/day groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Acceptable positive control data were provided.

No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. One female (#16197) was sacrificed in extremis on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material in the range finding study. An increased ($p \leq 0.01$) incidence of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females. During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of Compound Motor Action Potential (CMAP) (decreased 28%) that remained reduced, but to a lesser extent (decreased 13%), following the 6-week recovery period. Treatment-related neuropathological effects were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber

necrosis.

At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in 4/10 males and 3/10 females. These non-toxicological effects may have been caused by irritation of the oral mucosa.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

Dose Selected for Risk Assessment: NOAEL of 1.25 mg/kg/day.

Comments about Study/Endpoint:

Based on the observation in the 9-day female rat oral metabolism study (MRID 45774326), all the 4 mg/kg/day CuPT animals had irregular gait by Day 7 and paralysis by Day 9 at 0.5 and 4 hours post-dose. Therefore, the ADTC agreed that reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle can occur in short-term exposure condition and are appropriate for both short- and intermediate-term end-points.

4.3.3 Incidental Dermal - (Short- and Intermediate-term)

Study Selected: Dermal Developmental toxicity Rats

MRID No.: 46534001

Executive Summary: In a developmental toxicity study (MRID 46534001), zinc pyrithione (98.3% a.i., batch/lot # 0108244691) was dermally administered to 23-25 Crl:CD® (SD)IGS BR VAF/Plus® rats/dose at dose levels of 0, 10, 15, 30, or 60 mg/kg bw/day from day 0 through 20 of gestation.

Dermal administration of zinc pyrithione resulted in maternal toxicity at 30 and 60 mg/kg/day. Treatment-related effects at 60 mg/kg/day included clinical signs of toxicity (grade 1 erythema; grade 1 flaking; limited or no use of hindlimbs; shuffling gait; dehydration; low carriage; chromodacyorrhea; emaciation; chromorhinorrhea; hunched posture; decreased body weight and body weight gain, decreased uterine weight, decreased corrected body weight and corrected body weight gain, decreased absolute and relative feed consumption, and decreased muscle tone and mass.

At 30 mg/kg/day, there was an increase in the number of dams with limited use of hindlimbs, shuffling gait, decreased body weight and body weight gain, decreased corrected body weight and corrected body weight gain, and decreased absolute feed consumption. There were no treatment-related maternal effects at 10 or 15 mg/kg/day.

The maternal LOAEL is 30 mg/kg bw/day, based on an increase in the number of dams with limited use of hindlimbs, shuffling gait, decreased body weight and body weight gain, decreased corrected body weight and corrected body weight gain, and decreased absolute feed consumption. The maternal NOAEL is 15 mg/kg bw/day.

Developmental toxicity occurred at 60 mg/kg/day. Treatment-related findings at 60 mg/kg/day included decreased fetal weight (total, male, and female), an increase in the percentage of litters and fetuses with incomplete ossification of the sternal centra, an increase in the percentage of fetuses with wavy ribs, and a decrease in the ossification site averages for the caudal vertebrae, forelimb phalanges and metacarpals, and hindlimb phalanges and metatarsals per fetus per litter. There were no treatment-related developmental effects at 10, 15, or 30 mg/kg/day.

The developmental LOAEL is 60 mg/kg bw/day, based on decreased fetal weight (total, male, and female), an increase in the percentage of litters and fetuses with incomplete ossification of the sternal centra, an increase in the percentage of fetuses with wavy ribs, and a decrease in the ossification site averages for the caudal vertebrae, forelimb phalanges and metacarpals, and hindlimb phalanges and metatarsals per fetus per litter. The developmental NOAEL is 30 mg/kg bw/day.

Dose Selected for Risk Assessment: **NOAEL=15 mg/kg/day based on the maternal LOAEL of 30 mg/kg/day, where an increased number of dams with limited use of hindlimbs was observed, as well as observations of shuffling gait, decreased body weight and body weight gain, and decreased food consumption.**

Comments about Study/Endpoint: A 90-day dermal toxicity study was available for ZnPT (MRID 42827902) and was previously used for the dermal endpoint. However, the dermal developmental study demonstrated a lower NOAEL and is protective of effects occurring in developing mammalian organisms, as the endpoint is based upon effects in pregnant animals. Effects of ZnPT appear to differ in pregnant animals based on the available data comparing toxicity in pregnant vs. non-pregnant animals. Therefore, this study (MRID 46534001) is appropriate for dermal risk assessments.

4.3.4 Incidental Inhalation - (Short- and Intermediate-term)

Initially, inhalation toxicity was identified as a data gap for CuPT. However, with the submitted 28-day inhalation toxicity study for CuPT (MRID 48006403) and inhalation metabolism study (MRID 48006401), the ADTC concluded that the available information is adequate to address the issues associated with inhalation hazard concern for CuPT. In addition, other 28-day inhalation toxicity studies conducted with both ZnPT (MRID 48006404) and CuPT (MRID 48006403) addressed the issue of toxicological equivalence between ZnPT and CuPT for short and intermediate-term exposures. Therefore, the requirement of a 90-day inhalation study for CuPT can be waived. Based upon toxicological equivalence, the inhalation endpoint for CuPT is

established from the existing 90-day study conducted with ZnPT as shown below.

Study Selected: Subchronic Inhalation Rats

MRID No.: 42827903

Executive Summary: Groups of 15 male and 15 female Sprague Dawley rats were dynamically exposed in whole body exposure chambers to zinc pyrithione aerosols at concentrations of 0.0005, 0.0025, or 0.01 mg/L/day for 6 hours/day, 5 days/week for 13 weeks. One male and 1 female exposed to 0.0025 mg/L/day and 3 males and 4 females exposed to 0.01 mg/L/day died over the course of the study. Treatment related clinical signs of toxicity included rales, labored breathing, and gasping in animals that died on study. At 0.01 mg/L/day, body weights of females were depressed as much as 23%, compared to controls, total food consumption was decreased 10%, and food efficiency was decreased 53%. Hematologic, clinical chemistry, or urinalysis effects were not considered biologically significant for any of the exposure groups, and ophthalmologic examinations and gross necropsy findings were negative. Biologically significant increases in absolute and (relative) lung weights of +20% (+34%) and +22% (+38%) in males, and +13% (+18%) and +25% (+68%) in females were seen at concentrations of 0.0025 and 0.01 mg/L/day, respectively. The increased lung weights corresponded to histopathologic findings of trace to mild subacute inflammation of the interstitial tissue of the lung and medial hypertrophy of pulmonary arteries which were biologically significant at 0.01 mg/L/day. **NOAEL = 0.0005 mg/L/day. LOAEL = 0.0025 mg/L/day (labored breathing, rales, increased salivation, decreased activity, dry red brown material around the nose, increased absolute and relative lung weights, and death of undetermined cause).**

Comments about Study/Endpoint: The selected endpoint from the 90-day study will be used for short- and intermediate- risk assessments. A 21-day inhalation toxicity study is also available for ZnPT (MRID 46528101). This study demonstrated a LOAEL of 0.002 mg/L/day, similar to the LOAEL in the 90-day study, but did not test at lower concentrations as the 90-day study did. The effects observed in the 21-day study (gasping, respiratory gurgling, increased lung weight, histopathological changes in the lung) were similar to that of the 90-day study. Therefore, the 21-day study is considered supportive of the endpoint selected from the 90-day study.

Human Equivalent Concentration (HEC) Calculation :

For inhalation risk assessment, a human equivalent concentration (HEC) is calculated using the Agency's RfC guidance (USEPA, 1994: Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, publication # EPA/600/8-90/066F). Derivation of human equivalent concentrations reduces the magnitude of uncertainty and also provides a more direct comparison with estimated human exposures for calculation of inhalation risk Details of this derivation are provided below.

1) Step one: Adjustment of NOAEL/LOAEL

The NOAEL value selected for the exposure scenario of interest is first adjusted to reflect the

duration of the exposure scenario and to account for differences between the human exposure duration and that reported in experimental animal studies. For ZnPT inhalation risk assessments, short-, intermediate-, and long-term term occupational exposures are expected, as are short-term residential exposures. As the animal studies were conducted using a 6 hour exposure time, adjustment for an 8 hour work day for occupational assessments will be needed, and will also be sufficient for residential exposures, which are expected to be less than 8 hours.

Based on the above, the NOAEL is adjusted as follows for occupational and residential exposures:

NOAEL [ADJ] = 0.5 mg/m3 x (6hr/8hr) = 0.375 mg/m3

2) Step two: Calculation of Human Equivalent Concentration

Adjusted NOAEL or LOAEL values are used to calculate a human equivalent concentration (HEC) based on the general equation

NOAEL or LOAEL HEC = NOAEL or LOAEL [ADJ] x DAF

DAF is a dosimetric adjustment factor for the respiratory tract region, either the regional deposited dose ratio (RDDR) for particles or the regional gas dose ratio (RGDR) for gases.
In the case of ZnPT (an aerosol), the RDDR method is used to calculate the DAF. Based on the calculated RDDR, the HEC is calculated as:

0.375 mg/m3 x 1.57 [RDDR] = 0.58 mg/m3

3) Step three: Application of Uncertainty Factors for calculation of inhalation Reference Concentrations

For calculation of 'RfC' values for occupational and residential exposures, a total uncertainty factor of 30 is employed (3x for inter-species extrapolation, 10x for human variability). A 3x for inter-species extrapolation is used in place of the standard 10x factor as calculation of the RDDR incorporates dosimetric adjustments and therefore accounts for pharmacokinetic differences between animals and humans, leaving the 3x pharmacodynamic uncertainty component (USEPA, 1994).

5.0 RECOMMENDED MARGINS OF EXPOSURE

For incidental oral exposures, a margin of exposure (MOE) of 100 (10x for inter-species extrapolation, 10x for intra-species variation) is adequate for occupational exposure risk

assessments. A MOE of 100 (10x for inter-species extrapolation, 10x for intra-species variation) is also considered adequate for oral and dermal residential exposures to CuPT. For inhalation exposures, a MOE of 30 (3x for inter-species extrapolation, 10x for human variability) is adequate for inhalation risk assessments. The typical 10x inter-species scaling factor is reduced to 3x based on the calculation of an HEC.

6.0 AGGREGATE EXPOSURE RISK ASSESSMENTS

For short- and intermediate-term aggregate risk assessments, incidental oral and dermal exposures can be combined in determining the aggregate MOE based on similar toxicological effects observed after both oral and dermal exposure to pyrithiones. Inhalation exposures are not aggregated with incidental oral or dermal exposures, as the toxicologic response by this route differs from effects observed by oral or dermal exposure.

7.0 CARCINOGENIC/ MUTAGENICITY POTENTIAL

No chronic toxicity or carcinogenicity studies are available to assess the chronic toxicity or carcinogenicity of CuPT. There is a battery of negative mutagenicity studies.

8.0 FQPA CONSIDERATIONS

There are no food uses for CuPT. FQPA considerations do not currently apply to this chemical. It is noted; however, that the previous FQPA assessment for ZnPT in the October 21, 2010 ADTC memo identified no concerns for susceptibility, and there were sufficient data to characterize neurotoxicity of the chemical.

9.0 DATA GAPS

For exposure and risk assessments for antifoulant paint use, no data gaps were identified. However, if any risk assessments associated with long term exposure scenarios are needed, there are no chronic toxicity or carcinogenicity data available for CuPT. In addition, there is no reproductive toxicity study for CuPT. The lack of these data and the inability to bridge based on pharmacokinetic differences of CuPT compared to ZnPT and NaPT identify these areas as data gaps that would need to be satisfied if uses involving chronic exposures are sought.

10.0 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for CuPT up to intermediate-term exposures are summarized in Table 3.

Table 3 Toxicological Dose and Endpoints for Copper Pyrithione for use in human risk assessments for Antifoulant Paint Use.

Exposure Scenario	Dose Used in Risk Assessment/ UF	LOC for Risk Assessment	Study and Toxicological Endpoints
Acute Dietary	Endpoint is not required for CuPT as there are no food uses.		
Chronic Dietary	Endpoint is not required for CuPT as there are no food uses.		
Incidental Oral Short- and Intermediate-Term	Maternal NOAEL= 1.25 mg/kg/day	MOE = 100 (residential) MOE = 100 (occupational)	90-day Oral Toxicity Study-Rats (MRID 47023701) (CuPT) LOAEL = 2.25 mg/kg/day, based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle.
Dermal Short- and Intermediate -term	Dermal NOAEL = 15 mg/kg/day	MOE = 100 (residential) MOE = 100 (occupational)	Dermal Developmental Toxicity-Rats (MRID 46534001) (ZnPT) Maternal LOAEL = 30 mg/kg/day, based on increased no. of dame with limited use of hindlimbs, shuffling gait, decreased body weight and body weight gain, and decreased food consumption.
Inhalation Short and Intermediate-Term	Inhalation NOAEL = 0.0005 mg/L/day HEC = 0.58 mg/m^3	MOE = 30 (residential) MOE = 30 (occupational)	Subchronic Inhalation Toxicity-Rats (MRID 42827903) (ZnPT) LOAEL = 0.0025 mg/L Based on clinical signs of toxicity, decreased activity, and increased lung weights.

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TXR No. : 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D. **Signature:** Jonathan Chen
RASSB, Antimicrobials Division **Date:** 02/11/2011
Secondary Review: Timothy F. McMahon, Ph.D. **Signature:**
Senior Scientist, Antimicrobials Division **Date:** 2/15/11
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: 4-week Inhalation Toxicity - [rat] (registrant submitted protocol)

PC CODE: 088002 **DP BARCODE:** D375749

TEST MATERIAL (PURITY): Zinc Omadine® (98.3% a.i.)

SYNONYMS: Zinc pyrithione

CITATION: Kirkpatrick, D.T. (2009) A 4-week inhalation toxicity study with emphasis on pulmonary effects of zinc omadine in Sprague Dawley rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544008, December 14, 2009. MRID 48006404. Unpublished

SPONSOR: Arch Chemicals, Inc., 350 Knotter Drive, Cheshire, CT

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 48006404), Zinc Omadine® (98.3% a.i., Lot no. 0108244691) was administered as a dust aerosol to 15 Crl:CD(SD) rats/sex/concentration by nose-only exposure at concentrations of 0 (air), 0.5, 1.5, or 5.0 mg/m^3 (equivalent to analytical concentrations of 0, 0.0005, 0.0015, and 0.0051 mg/L) for 6 hours per day, 5 days/week for up to 4 weeks (up to 20 exposures). Five rats/sex/concentration were euthanized following 1, 2, and 4 weeks of exposure and subjected to a gross necropsy. Selected organs were weighed and examined microscopically. A special emphasis was placed on the evaluation of pulmonary effects, including assessment of bronchoalveolar lavage fluid (BALF) parameters and microscopic examination of the lungs.

At 0.005 mg/L, one female (No. 5638) was found dead on Day 15. Histopathology findings consisted of moderate congestion in the lungs, minimal increased mucous in the respiratory epithelium, and mild perivascular inflammation, as well as mild degeneration of skeletal muscle. The findings in the lungs were similar in degree and nature to the findings in other treated animals. The cause of death was not definitively determined. It was stated that the microscopic findings may have contributed to the death of this animal, but were not the primary cause. Clinical signs of toxicity observed in this animal prior to its death included thinness, dermal atonia, and body weight loss. In the surviving females in this group, treatment-related clinical signs of toxicity included thin body condition in three females and impaired use of the hindlimbs in one female on Day 24 at the 0 to 1 hour post-exposure examination. One of these animals lost 68 grams during Days 18-25 and was noted as hypothermic on Day 26.
In the 0.005 mg/L males, body weights were significantly ($p<0.01$) decreased by 7-15%

throughout the study compared to controls. A body weight loss was observed for Days 0-4 at this concentration (-6 g) compared to a body weight gain in the controls (13 g). Body weight gain for Days 4-11 was decreased by 31% ($p<0.01$), and cumulative body weight gains were decreased ($p<0.01$) by 51-71% in the 0.005 mg/L males for each of the remaining study intervals.

In the 0.005 mg/L females, body weights were decreased by 4-17% (NS) throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 49-89% lower ($p<0.05$) than controls for each of the intervals throughout the study, and a body weight loss was noted for Days 18-25 (-14 g) compared to a weight gain in the controls (14 g).

Food consumption was dose-dependently decreased by 11-29% ($p<0.01$) in both sexes and by 8-16% ($p<0.05$) in the males for Days 4-11 at 0.0015 and 0.005 mg/L.

Treatment-related effects on BALF parameters were noted in all treated groups in both sexes. These effects included increased lactate dehydrogenase (LDH) and total protein, higher percentages of eosinophils, neutrophils, and lymphocytes, and lower percentages of alveolar macrophages. Mean LDH and total protein levels were higher than control means for all exposure levels on Days 5, 12, and 26, with concentration-related trends observed in the females but not the males. For LDH, intra-group variability was high and statistical significance was limited to the 0.005 mg/L females on Day 5. For total protein, statistical significance was attained in the 0.0015 mg/L females (Day 26) and in the 0.005 mg/L males (Days 5 and 26) and females (Days 5, 12, and 26). For bronchoalveolar lavage cytology, the proportion of eosinophils was increased over controls in a concentration-dependent manner in both sexes at all treatment levels. With the exception of the 0.0005 mg/L males on Day 12, the percentages of eosinophils were highest on Day 5, with progressively lower counts on Days 12 and 26. The higher percentage of eosinophils was more pronounced in females at all exposure levels and at all intervals when compared to males. The proportions of neutrophils and lymphocytes in all treated groups were minimally higher than controls in both sexes. Associated with these changes, the proportions of alveolar macrophages were lower in all treated groups. With the minor exception of the females on Day 26, the decreases in the percent alveolar macrophages were concentration-dependent.

Lung weights (absolute, relative to body weight, and relative to brain weight) were increased in both sexes in all treated groups throughout the study compared to controls. With the single exception of the lung weights relative to brain weight on Day 26 in the males, these increases were concentration-dependent. Statistical significance ($p<0.05$) was attained at 0.0005 mg/L in the males on Day 26 and in the females on Days 12 and 26. The increases at 0.0015 and 0.005 mg/L were statistically significant ($p<0.05$) in both sexes at each of the intervals, with the exception of the lung weight relative to brain weight in the 0.0015 mg/L males on Day 26.

Thymus weights (absolute, relative to body weight, and relative to brain weight) were decreased by 24-40% at 0.005 mg/L compared to controls in both sexes throughout the study. The investigators stated that decreases were most likely secondary effects related to stress.

At 0.005 mg/L, dark red areas in the lungs were observed in the female that died and in the females terminated on schedule on Days 5 (1/5) and 26 (1/4). Dark red areas in the lungs were also noted in one male at 0.0015 mg/L on Day 5. Enlarged bronchial lymph nodes were found in the 0.005 mg/L females on Day 5 (2/5) and Day 12 (1/5) compared to 0 controls. Enlarged mediastinal lymph nodes were found in the 0.0015 mg/L males on Day 12 (1/5), in the 0.0015 mg/L females on Day 26 (1/5), and in the 0.005 mg/L females on Day 26 (1/5).

Treatment-related microscopic findings in the lungs were observed in all groups exposed to the test material compared to no findings in the controls. In the lungs, minimal to moderate subacute inflammation was observed at 0.0005 mg/L (1/15 males; 5/15 females), 0.0015 mg/L (8/15 males; 13/15 females), and 0.005 mg/L (3/15 males; 7/14 females). Minimal to moderate alveolar macrophages were observed at 0.0005 mg/L (2/15 males; 3/15 females), 0.0015 mg/L (11/15 males; 1/15 females), and 0.005 mg/L (15/15 males; 12/14 females). Minimal to moderate perivasculitis was found at 0.0005 mg/L (11/15 males; 14/15 females), 0.0015 mg/L (13/15 males; 15/15 females), and 0.005 mg/L (15/15 males; 14/14 females). Minimal to moderate hypertrophy of the smooth muscle of the alveolar ducts of the lungs was noted at 0.0005 mg/L (4/14 males; 6/13 females), 0.0015 mg/L (6/13 males; 7/15 females), and 0.005 mg/L (13/15 males; 13/14 females). These findings were considered to be due to direct effects of the test material on the respiratory tract.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to mild degeneration (2/14 treated vs 0/15 controls) There were no other treatment-related findings in the organs and tissues examined microscopically.

The LOAEL is 0.0005 mg/L based on increases in BALF parameters (increased LDH, total protein, and proportions of neutrophils and lymphocytes), lung weights, and incidences of microscopic findings in the lungs (subacute inflammation, alveolar macrophages, perivasculitis, and hypertrophy of the smooth muscle of the alveolar ducts). A NOAEL was not established.

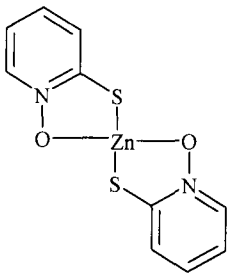
This 28-day study is classified as **acceptable/non-guideline.** This study was conducted after review of a protocol submitted by the registrant as part of discussions between the Antimicrobials Division and the registrant to determine toxic similarity between zinc pyrithione and copper pyrithione. The purpose of this study with zinc pyrithione was to examine toxic effects of zinc pyrithione by inhalation after 4 weeks, which included examination of lung bronchioalveolar lavage fluid after single or repeated inhalation exposures, and examination of lung histopathology. Certain parameters (microscopic examination of nasal passages, trachea, and larynx; neurobehavioral, ophthalmologic, and clinical pathology examinations) were not conducted in this study. A similar study has been conducted with copper pyrithione.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Zinc Omadine®

Description:	Uniform off-white powder
Lot #:	0108244691
Purity:	98.3% a.i.
Compound stability:	Samples of the test article were collected and analyzed prior to use on the study (93% pure) and after use on the study 40 days later (88.0% pure). Furthermore, concentration analyses were determined for each test atmosphere at least twice during each exposure period. The results of these analyses, shown in Table 1 of this DER, indicated that the animals were exposed to a consistent test concentration over the duration of the study.
CAS # of TGAI:	13463-41-7
Structure:	

2. Vehicle and/or positive control: Filtered air

3. Test animals

Species:	Rat	
Strain:	Crl:CD(SD)	
Age/weight at study initiation:	Approximately 8 weeks old; 229-273 g males, 167-211 g females	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	Individually in stainless steel wire mesh cages suspended above cage board	
Diet:	Certified Rodent LabDiet 5002 (PMI Nutrition International, St. Louis, MO), *ad libitum*, except during exposure and overnight prior to termination	
Water:	Reverse-osmosis filtered (on-site) water, *ad libitum*, except during exposure	
Environmental conditions:	**Temperature:**	21.3-21.8EC
	Humidity:	41.2-52.2%
	Air changes:	At least 10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN

1. In life dates: Start: July 15, 2009 End: August 11, 2009

2. Animal assignment: During the pre-test period, animals were acclimated to restraint in nose-only exposure restraint tubes by increasing the restraint time over the acclimation period (one hour on the first day, 2 hours on the second day, 3 hours on the third day, four hours on the fourth day, and six hours on the fifth day). Two days (for males) or three days (for females) prior to the initiation of exposures, animals deemed acceptable for inclusion in the study (based on appropriate food consumption and body weight gain, acclimation to the nose-only restraint system, and lack of physical/clinical abnormalities) were randomly assigned,

stratified by body weight, to the test groups noted in Table 1. Individual body weights at randomization were within ± 20% of the mean body weight for each sex.

TABLE 1: Study design [a]

Test group	Target conc. (mg/m^3)	Analytical conc. (mg/m^3)	Analytical conc. (mg/L) [b]	MMAD Φm	GSD	Rats/sex
Control	0	0	0	0	0	15
Low (LCT)	0.5	0.5	0.0005	1.3	2.00	15
Mid (MCT)	1.5	1.5	0.0015	1.8	2.30	15
High (HCT)	5.0	5.1	0.0051	1.8	2.03	15

a Data were obtained from page 16 and Text Tables 1, 2, and 3 on pages 36 and 37 of the study report.
b Analytical concentrations were converted from mg/m^3 to mg/L by the reviewers by dividing by 1000.

3. **Concentration selection rationale:** It was stated that the exposure concentrations were selected based upon known toxicity information, including the results of a 5-day range-finding study (WIL-544006; Kirkpatrick, Draft). No further information was provided.

4. **Test material administration:** The test substance was administered as a dust aerosol via nose-only inhalation for 6 hours per day, 5 days per week for up to 4 consecutive weeks (up to 20 exposure days).

5. **Generation of the test atmosphere / chamber description:** A diagram of the test atmosphere generation system and exposure chamber was included as Figure 1 on page 597 of the study report. This figure is included in the Appendix in this DER.

   Exposures were conducted using an 11.0-L or 14.1-L conventional nose-only exposure system (designed and fabricated by WIL Research Laboratories, LLC) with synthetic rubber grommets in exposure ports to engage animal holding tubes. One exposure system was dedicated for each group for the duration of the study. Air supplied to the nose-only system was provided from a dry compressed air source. All test atmosphere exhaust passed through the facility exhaust system, which consisted of charcoal- and HEPA-filtration. Exposure chamber temperature, relative humidity, and chamber ventilation rate were continually monitored and manually recorded at approximately 60-minute intervals during the exposure. The mean temperature and mean relative humidity were set for 19-25°C and 30-70%, respectively. All exposure systems were operated under dynamic conditions, with at least 12 air changes per hour, at a slight negative pressure. Oxygen content for the test substance chambers was measured during the method development phase of the study and was at least 19%.

   A dust aerosol atmosphere of the test substance was generated using a Wright Dust Feeder (WDF) and controller, which delivered the test substance aerosol at a constant rate to a stainless steel distribution drum. Dry compressed air was supplied to the WDF. The aerosol from the distribution drum was directed to each test substance exposure system using a transvector jet. Control animals were exposed to filtered air using an exposure regimen equivalent to the test substance exposures.

Test atmosphere concentrations – Actual exposure concentrations were determined using standard gravimetric methods. Samples were collected on pre-weighed, 25-mm glass-fiber filters held in open-faced filter holders positioned in the animal breathing zones of the nose-only exposure systems. Following sample collection, the filter was re-weighed and the concentration calculated as the filter weight difference divided by the sample volume. One sample was collected weekly for the control exposure system and 2, 3, and 6 samples were collected per exposure day for exposure systems 2, 3, and 4, respectively.

Particle size determination – Aerosol particle size determinations were conducted for each test substance exposure system using a 7-stage cascade impactor. At least one sample was collected weekly for each test substance exposure system. For each test substance exposure system, one supplemental particle size determination was performed using an Anderson cascade impactor.

6. **Statistics:** Body weight, body weight change, food consumption, organ weight data, and bronchoalveolar lavage fluid total protein and LDH values were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) differences among groups, Dunnett's test was used to compare each treated group with the controls. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. **Cageside observations:** All animals were observed for mortality and moribundity twice daily (once in the morning and once in the afternoon).

b. **Clinical examinations:** Clinical examinations were performed prior to exposure, 0 to 1 hour following exposure (designated as 1 hour post-exposure for report presentation purposes), and once daily on non-exposure days. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were conducted on all animals at least once during the pretest period, at the time of randomization and group assignment, and weekly during the exposure phase (including prior to the scheduled necropsies). On days when detailed physical examinations were conducted, clinical observations were only performed at the post-exposure time point.

2. **Body weight:** Individual body weights were recorded at least weekly during the pretest period, at the time of randomization and group assignment, and prior to the first exposure. During the exposure period, individual body weights were recorded prior to exposure on Days 4, 11, 18, and 25. Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded on the day of the scheduled necropsies.

3. **Food consumption:** Individual food consumption was recorded at least weekly during the pretest period and throughout the study. Mean food consumption was calculated as g/animal/day for the corresponding body weight intervals. When food consumption could not be measured for a given interval (due to spillage, weighing error, obvious erroneous value, etc.), the appropriate interval was footnoted as "NA" (Not Applicable) in the summary tables.

4. **Ophthalmoscopic examination:** Ocular examinations were not conducted.

5. **Hematology and clinical chemistry:** Not conducted.

6. **Urinalysis**: Not conducted.

7. **Bronchoalveolar lavage fluid (BALF) evaluation:** During the scheduled necropsy evaluations on Days 5, 12, and 26, 5 animals/sex/concentration were anesthetized using isoflurane inhalation and euthanized by exsanguination. As soon as possible after exsanguination, the lungs and trachea were removed, weighed, and a ligature was placed on the left mainstem bronchus. BALF samples were obtained via lavage of the right lung, and the following parameters were evaluated:

Total cell count for alveolar macrophages [a]
Differential cell count for alveolar macrophages [b]
Neutrophils
Lymphocytes
Eosinophils
Basophils
Epithelial cells
Lactate dehydrogenase (LDH) [c]
Total protein [c]

a Performed by WIL Research Laboratories, LLC using a hemocytometer
b Performed by Gail L. Walter, MT(ASCP), DVM, DACVP, DABT, using stained Cytospin slides prepared by WIL Research Laboratories, LLC
c Performed at WIL Research Laboratories, LLC using a Hitachi 912 Chemistry Analyzer

8. **Pathology:** After completion of the BALF evaluation, the clamp was removed from the left mainstem bronchus, and the lungs were fixed by constant pressure inflation with fixative. A complete necropsy was conducted for all animals. The following CHECKED (X) tissues were collected, fixed in 10% neutral-buffered formalin (except as noted), processed routinely, and stained with hematoxylin and eosin. Following collection of the appropriate protocol-specified tissues, the entire head was removed and preserved. After decalcification, six cross-sections of the nasal cavities were prepared for microscopic examination in accordance to the methods described by Morgan (1991). Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*±
X	Salivary glands*	XX	Heart*±	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*±	X	Eyes (optic nerve)* [b]
X	Jejunum*	XX	Thymus*±		GLANDULAR
X	Ileum*			XX	Adrenal gland*±
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*±	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*±	XX	Testes*± [a]		OTHER
	Gall bladder* (not rat)	XX	Epididymides*± [a]	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin
	RESPIRATORY	XX	Ovaries (and oviducts)*±	X	All gross lesions and masses*
X	Trachea*	XX	Uterus (with cervix)*±	X	Harderian gland
XX	Lungs*	X	Mammary gland (females)* [c]	X	Peyer's patches
X	Nasal cavities* [d]	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465
± Organ weights required
a Fixed in Bouin's solution
b Fixed in Davidson's solution
c A corresponding section of skin was collected from the same anatomic location for males.
d After decalcification, six cross-sections of the nasal cavities were prepared for microscopic examination in accordance to the methods described by Morgan (1991).

Microscopic examinations were performed on the lungs, liver, kidneys, brain, stomach, skeletal muscle, and gross lesions of all animals. A formal pathology peer review was conducted on tissues from the brain, lungs, skeletal muscle, and gross lesions from all animals.

II. RESULTS

A. OBSERVATIONS

1. Test material exposure and characterization

The test report notes on page 586 that "Exposures were conducted using an 11.0L or 14.1L conventional nose-only exposure system…with synthetic rubber groments in exposure ports to engage animal holding tubes. Exposure systems 2 and 3 were 4-tier (14.1L) nose-only systems to accommodate exposure of animals from the WIL-544011 metabolism study (submitted as MRID 48006402) to the unlabeled test substance on various exposure days."

The table below summarizes the mean exposure concentration for each dose group, as well as particle size measurements (MMAD and GSD) for each dose group.

Test Concentrations

Dose group (males)	Target Concentration (mg/L)	Mean Concentration (mg/L)	Mean MMAD (µm)	Mean GSD (µm)
Control	0	0	0	0
Low	0.5	0.52	1.3	2.00
Mid	1.5	1.5	1.8	2.30
High	5.0	5.1	1.8	2.03

2. **Mortality:** At 0.005 mg/L, one female (No. 5638) was found dead on Day 15. Histopathology findings consisted of moderate congestion in the lungs, minimal increased mucous in the respiratory epithelium, and mild perivascular inflammation, as well as mild degeneration of skeletal muscle. The findings in the lungs were similar in degree and nature to the findings in other treated animals. The cause of death was not definitively determined. It was stated that the microscopic findings may have contributed to the death of this animal, but were not the primary cause. All other animals survived until scheduled termination.

3. **Clinical signs of toxicity:** Clinical signs of toxicity observed in female No. 5638 prior to its death on Day 15 included thinness, dermal atonia, and a cumulative body weight loss of 29 g (from Days 0-11). In the surviving females in this group, treatment-related clinical signs of toxicity included thin body condition in three females (Nos. 5638, 5649, and 5666) and impaired use of the hindlimbs in one female (No. 5649) on Day 24 at the 0 to 1 hour post-exposure examination. Female No. 5649 was also noted as hypothermic (body and extremities cool to the touch) on Day 26. This animal lost 68 grams during Days 18-25. There were no other treatment-related clinical observations.

B. **BODY WEIGHT AND WEIGHT GAIN:** Body weight and body weight gain data are presented in Table 2. Treatment-related effects were observed in both sexes at 0.005 mg/L.

In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased by 7-15% throughout the study compared to controls A body weight loss was observed for Days 0-4 at this concentration (-6 g) compared to a body weight gain in the controls (13 g). Body weight gain for Days 4-11 was decreased by 31% ($p \leq 0.01$), and cumulative body weight gains were decreased ($p \leq 0.01$) by 51-71% in the 0.005 mg/L males at each of the remaining study intervals.

In the 0.005 mg/L females, body weights were decreased by 4-17% (not significant [NS]) throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 49-89% lower ($p \leq 0.05$) than controls at each of the intervals throughout the study, and a body weight loss was noted for Days 18-25 (-14 g) compared to a weight gain in the controls (14 g).

There were no other adverse effects of treatment on body weights or body weight gains. In the 0.0015 mg/L males and females, cumulative body weight gains were decreased by 14-33% throughout the study. However, these decreases were not statistically significant and did not result in statistically or biologically significant decreases in body weights (<8%).

TABLE 2. Mean (∀SD) body weights and body weight gains (g) during 26 days of treatment with Zinc Omadine via inhalation [a]

Day(s)	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Males				
0	262±11.8	262±10.3	262±11.2	263±11.6
4	275±14.6	273±15.3	271±13.4	257±12.1** (↓7)
11	306±16.8	307±16.6	292±15.0	272±18.1** (↓11)
18	338±15.8	331±17.9	314±15.7	293±26.1** (↓13)
25	361±22.4	357±23.6	338±17.0	308±33.6** (↓15)
0-4	13±4.7	11±7.2	9±5.1	-6±3.9**
4-11	26±4.6	31±2.6	24±5.9	18±9.1** (↓31)
0-11	41±8.0	43±7.1	32±8.8 (↓22)	12±12.8** (↓71)
0-18	68±5.8	68±10.8	55±11.3 (↓19)	31±18.6** (↓54)
0-25	92±13.6	94±14.0	79±11.8 (↓14)	45±25.6** (↓51)
Females				
0	196±11.6	194±10.5	197±12.6	194±13.8
4	205±13.3	203±12.7	203±12.4	196±15.0 (↓4)
11	218±12.4	217±18.9	214±16.1	206±25.2 (↓6)
18	240±13.6	232±17.6	224±18.8	226±14.9 (↓6)
25	254±12.7	244±14.6	234±18.0	212±47.2 (↓17)
0-4	9±4.2	9±4.5	6±3.1	1±6.2**
18-25	14±2.5	12±5.8	10±2.7	-14±36.2
0-11	22±6.1	23±8.3	18±5.2 (↓18)	10±16.6* (↓55)
0-18	41±8.4	35±8.5	31±5.3 (↓24)	21±5.2** (↓49)
0-25	55±7.3	47±9.4	41±6.0 (↓25)	6±37.9** (↓89)

a Data obtained from Tables 11 through 16 on pages 80-89 in the study report. n = 15 for Day 0 and 4, n = 10 for Day 11, and n = 5 for Days 18 and 25 (except for the 0.005 mg/L females, where n = 4 for Days 18 and 25).

* Statistically different from the control group at $p < 0.05$

** Statistically different from the control group at $p < 0.01$

C. FOOD CONSUMPTION

1. **Food consumption:** Food consumption data are presented in Table 3. Food consumption was dose-dependently decreased ($p \leq 0.01$) in both sexes at 0.0015 mg/L (↓11-13%) and 0.005 mg/L (↓26-29%) for Days 0-4. Additionally in the males, food consumption was decreased by 8-16% ($p < 0.05$) at 0.0015 and 0.005 mg/L for Days 4-11. Food consumption in the

0.0005 mg/L males and females was comparable to controls throughout the study.

TABLE 3. Mean (±SD) food consumption (g/animal/day) during 28 days of treatment with Zinc Omadine via inhalation[a]

Day(s)	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Males				
-8 to -2	25±1.5	24±1.4	25±1.0	25±1.3
N	15	15	15	15
0 to 4	24±2.0	23±1.9	21±1.6** (↓13)	17±1.0** (↓29)
N	15	15	15	15
4 to 11	25±1.9	24±1.5	23±1.8* (↓8)	21±1.8** (↓16)
N	10	10	10	10
11 to 18	26±1.9	25±1.9	24±1.9	23±1.5
N	5	5	5	5
18 to 25	26±2.8	26±2.1	25±2.2	23±2.6
N	5	5	5	5
Females				
-9 to -3	19±1.7	18±1.6	19±2.4	19±2.0
N	15	15	15	14
0 to 4	19±1.8	18±1.8	17±1.1** (↓11)	14±1.8** (↓26)
N	15	15	14	15
4 to 11	19±2.0	18±1.9	18±1.1	17±2.2
N	10	10	9	10
11 to 18	21±1.9	19±1.8	20±1.4	19±0.6
N	5	5	4	4
18 to 25	21±2.2	20±2.1	21±2.9	17±5.6
N	5	5	5	4

a Data were obtained from Tables 17 and 18 on pages 90 and 91 of the study report. Percent differences from the controls are included in parentheses.
* Statistically different from the control group at $p < 0.05$
** Statistically different from the control group at $p < 0.01$

D. SACRIFICE AND PATHOLOGY

1. **Bronchoalveolar lavage fluid (BALF):** Treatment-related effects on BALF parameters were noted in all treated groups in both sexes (Tables 4 through 7). These effects included increased LDH in the males (↑9-176%) and females (↑26-483%), increased total protein in the males (↑31-174%) and females (↑59-421%), higher percentages of eosinophils, neutrophils, and lymphocytes, and a lower percentage of alveolar macrophages.

   Mean LDH and total protein levels were higher than control means for all exposure levels on Days 5, 12, and 26, with concentration-related trends observed in the females but not the males. For LDH, intra-group variability was high and statistical significance was limited to the 0.005 mg/L females on Day 5. For total protein, statistical significance was attained in the 0.0015 mg/L females (Day 26) and in the 0.005 mg/L males (Days 5 and 26) and females

(Days 5, 12, and 26).
For bronchoalveolar lavage cytology, the most prominent and consistent change compared to controls was a concentration-dependent increase in the proportion of eosinophils in both sexes at all treatment levels. With the exception of the 0.0005 mg/L males on Day 12, the percentages of eosinophils were highest on Day 5, with progressively lower counts on Days 12 and 26. The higher percentage of eosinophils was more pronounced in females at all exposure levels and at all intervals when compared to males.

The proportions of neutrophils and lymphocytes in all treated groups were minimally higher than controls in both sexes. Associated with these changes, the proportions of alveolar macrophages were lower in all treated groups. The decreases in the percentage of alveolar macrophages were concentration-dependent, with the exception of the females on Day 26.

TABLE 4. Mean (∀SD) bronchiolar lavage fluid (BALF) absolute values in males during 28 days of treatment with Zinc Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Lactate dehydrogenase (U/L)				
Day 5	203±48.12	395.8±102.47 (↑95)	270.2±99.34 (↑33)	277.3±138.27 (↑37)
Day 12	152.9±95.71	343.8±222.7 (↑125)	421.9±235.9 (↑176)	224.0±46.65 (↑47)
Day 26	91.6±87.07	100.2±32.05 (↑9)	107.9±58.80 (↑18)	156.2±32.38 (↑71)
Total protein (mg/dL)				
Day 5	21.9±4.38	38.1±7.06 (↑74)	37.3±15.49 (↑70)	46.2±16.85* (↑111)
Day 12	17.5±7.73	36.6±22.62 (↑109)	47.9±29.00 (↑174)	31.8±3.57 (↑82)
Day 26	11.8±6.94	17.6±5.32 (↑49)	15.4±7.21 (↑31)	30.6±3.81** (↑159)
Total cell count (x 10^6)				
Day 5	11.43±2.133	7.37±1.733	12.35±4.746	6.96±2.869
Day 12	12.92±7.600	10.08±4.882	11.48±5.212	12.88±4.082
Day 26	17.48±10.226	16.73±8.202	9.13±6.393	14.76±5.107
Alveolar macrophages (x 10^6)				
Day 5	11.13±1.971	6.73±1.400** (↓39)	9.34±2.558 (↓16)	4.45±1.660** (↓60)
Day 12	12.70±7.570	8.59±3.535 (↓32)	9.16±5.164 (↓28)	9.98±4.119 (↓21)
Day 26	17.19±10.000	15.65±7.448 (↓9)	8.22±5.614 (↓52)	13.01±4.741 (↓24)
Neutrophils (x 10^6)				
Day 5	0.06±0.078	0.12±0.204 (↑100)	0.32±0.528 (↑433)	0.19±0.160 (↑216)
Day 12	0.04±0.043	0.21±0.207 (↑425)	0.60±0.375* (↑1400)	0.35±0.367 (↑775)
Day 26	0.06±0.077	0.10±0.161 (↑67)	0.21±0.368 (↑250)	0.11±0.150 (↑83)
Lymphocytes (x 10^6)				
Day 5	0.17±0.159	0.19±0.169 (↑12)	0.46±0.363 (↑171)	0.29±0.246 (↑71)
Day 12	0.12±0.077	0.31±0.310 (↑158)	0.53±0.253 (↑342)	0.55±0.425 (↑358)
Day 26	0.19±0.239	0.23±0.242 (↑21)	0.17±0.156 (↓11)	0.33±0.237 (↑74)
Eosinophils (x 10^6)				
Day 5	0.00±0.000	0.30±0.519	2.10±3.374	1.93±1.606
Day 12	0.00±0.000	0.93±1.616	1.07±0.699	1.84±0.838
Day 26	0.00±0.000	0.48±0.593	0.35±0.444	1.23±1.277

a Data were obtained from Table 19 on pages 92-98 in the study report. n = 5 for each time point.
* Statistically different from the control group at $p < 0.05$
** Statistically different from the control group at $p < 0.01$

TABLE 5. Mean (∀SD) bronchiolar lavage fluid (BALF) absolute values in females during 28 days of treatment with Zinc Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Lactate dehydrogenase (U/L)				
Day 5	173.9±105.30	271.1±110.35 (↑56)	425.2±154.95 (↑145)	1013.3±645.29** (↑483)
Day 12	171.2±119.20	215.4±112.42 (↑26)	250.1±104.13 (↑46)	376.5±284.35 (↑120)
Day 26	129.5±52.29	196.7±45.62 (↑52)	194.3±70.18 (↑50)	279.9±130.94 (↑116)
Total protein (mg/dL)				
Day 5	19.2±6.82	30.5±11.16 (↑59)	73.7±72.78 (↑284)	100.1±52.18* (↑421)
Day 12	14.3±9.06	35.7±19.49 (↑150)	47.6±13.69 (↑233)	51.5±30.64* (↑260)
Day 26	17.1±3.68	28.5±4.79 (↑67)	32.6±5.68* (↑91)	35.1±16.16* (↑105)
Total cell count (x 10^6)				
Day 5	8.09±3.355	9.33±4.275	12.61±7.454	11.39±4.356
Day 12	8.05±4.951	10.93±4.863	12.97±5.973	12.66±5.865
Day 26	10.19±3.860	8.09±4.246	9.57±2.144	5.82±2.090
Alveolar macrophages (x 10^6)				
Day 5	8.00±3.317	7.88±3.326	5.96±2.886	6.51±3.925
Day 12	7.93±4.910	8.74±3.031	9.66±4.875	7.62±2.307
Day 26	9.94±3.803	6.59±3.572	8.06±2.591	4.40±1.780
Neutrophils (x 10^6)				
Day 5	0.03±0.31	0.11±0.074	2.37±5.020	0.38±0.235
Day 12	0.01±0.018	0.40±0.543	0.37±0.334	0.50±0.825
Day 26	0.00±0.000	0.27±0.387	0.09±0.079	0.32±0.198
Lymphocytes (x 10^6)				
Day 5	0.04±0.026	0.27±0.219	0.73±0.667	0.77±0.503* (↑1825)
Day 12	0.10±0.096	0.44±0.490	0.80±0.400	0.49±0.422
Day 26	0.12±0.107	0.41±0.342	0.49±0.390	0.32±0.198
Eosinophils (x 10^6)				
Day 5	0.01±0.031	1.04±1.006 (↑10,300)	3.37±3.512* (↑33,600)	3.67±1.455* (↑36,600)
Day 12	0.01±0.027	1.29±1.483 (↑12,800)	2.07±1.646 (↑20,600)	3.69±2.547** (↑36,800)
Day 26	0.00±0.000	0.65±0.619	0.78±0.559	0.72±0.310

a Data were obtained from Table 20 on pages 103-109 in the study report. n = 5 for each time point, except n = 4 for 0.005 mg/L on Day 26.

* Statistically different from the control group at $p < 0.05$

** Statistically different from the control group at $p < 0.01$

TABLE 6. Mean (∀SD) bronchiolar lavage fluid (BALF) percent values in males during 28 days of treatment with Zinc Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Alveolar macrophages (%)				
Day 5	97.6±1.98	92.1±8.00	80.2±19.19 (↓18)	66.2±12.83** (↓32)
Day 12	97.5±2.32	86.8±11.51	76.0±14.65* (↓22)	75.9±12.04* (↓22)
Day 26	98.5±0.79	94.2±3.21	91.1±6.17 (↓8)	88.2±8.43* (↓11)
Neutrophils (%)				
Day 5	0.5±0.61	1.5±2.24 (↑200)	2.1±2.58 (↑320)	3.1±2.38 (↑520)
Day 12	0.3±0.27	2.1±1.60 (↑600)	6.1±4.02** (↑1933)	2.8±3.05 (↑833)
Day 26	0.4±0.65	0.5±0.61 (↑25)	1.8±2.02 (↑350)	0.9±1.24 (↑125)
Lymphocytes (%)				
Day 5	1.4±1.29	2.6±2.22 (↑86)	3.6±1.92 (↑157)	4.5±3.14 (↑221)
Day 12	1.6±2.22	2.9±2.27 (↑81)	5.2±2.44 (↑225)	4.9±4.92 (↑206)
Day 26	0.9±0.65	1.4±1.08 (↑55)	2.1±1.64 (↑133)	2.3±1.15 (↑156)
Eosinophils (%)				
Day 5	0.0±0.00	3.5±5.34	12.6±16.53	23.9±14.10*
Day 12	0.0±0.00	7.4±9.24	11.2±9.78	15.0±6.41*
Day 26	0.0±0.00	2.7±2.66	3.2±3.05	8.1±8.23

a Data were obtained from Table 19 on pages 99-102 in the study report. n = 5 for each time point.
* Statistically different from the control group at $p < 0.05$
** Statistically different from the control group at $p < 0.01$

TABLE 7. Mean (∀SD) bronchiolar lavage fluid (BALF) percent values in females during 28 days of treatment with Zinc Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Alveolar macrophages (%)				
Day 5	98.9±0.65	85.3±6.83 (↓14)	59.0±30.95** (↓40)	54.9±15.11** (↓45)
Day 12	98.4±1.19	83.4±12.19 (↓15)	73.8±11.76** (↓25)	64.5±12.61** (↓35)
Day 26	97.4±2.33	80.7±10.69* (↓17)	83.1±11.92 (↓15)	75.1±5.45** (↓23)
Neutrophils (%)				
Day 5	0.3±0.27	1.3±0.76 (↑333)	10.0±19.31 (↑3233)	3.5±1.84 (↑1067)
Day 12	0.1±0.22	2.7±3.15 (↑2600)	4.2±4.13 (↑4100)	3.1±3.75 (↑3000)
Day 26	0.0±0.00	2.4±3.05	1.1±0.96	5.1±1.44**
Lymphocytes (%)				
Day 5	0.5±0.35	2.9±2.10 (↑480)	5.4±2.04* (↑980)	7.1±3.96** (↑1320)
Day 12	1.1±1.19	3.5±2.24 (↑218)	6.4±1.64** (↑482)	3.6±2.10 (↑227)
Day 26	1.4±1.14	6.3±4.86 (↑350)	5.2±4.02 (↑271)	5.1±1.44 (↑264)
Eosinophils (%)				
Day 5	0.1±0.22	10.3±5.83 (↑10200)	23.8±15.04**	33.5±10.78**
Day 12	0.1±0.22	9.8±11.54 (↑9700)	15.0±8.54* (↑14,900)	26.6±7.26** (↑26,500)
Day 26	0.0±0.00	7.2±4.28	9.0±7.44*	12.8±4.84**

a Data were obtained from Table 20 on pages 110-113 in the study report. n = 5 for each time point.
* Statistically different from the control group at $p < 0.05$
** Statistically different from the control group at $p < 0.01$

2. **Organ weights:** Lung weights (absolute, relative to body weight, and relative to brain weight) were increased in both sexes throughout the study at 0.0005 mg/L (↑10-23%), 0.0015 mg/L (↑11-44%), and 0.005 mg/L (↑18-62%) compared to controls (Table 8). With the single exception of the lung weights relative to brain weight on Day 26 in the males, these increases were concentration-dependent. Statistical significance ($p \leq 0.05$) was attained at 0.0005 mg/L in the males on Day 26 and in the females on Days 12 and 26. The increases at 0.0015 and 0.005 mg/L were statistically significant ($p \leq 0.05$) in males and females at each of the intervals, with the exception of the lung weight relative to brain weight in the 0.0015 mg/L males on Day 26.

   Thymus weights (absolute, relative to body weight, and relative to brain weight) were decreased by 24-40% at 0.005 mg/L compared to controls in both sexes throughout the study (Table 9). The investigators stated that these decreases were most likely secondary effects related to stress.

   There were no other differences in organ weights that could be attributed to treatment. All other observed differences from controls were unrelated to concentration and/or were attributed to the decreased terminal body weights at 0.005 mg/L in the males on Days 12 and 26 and in the females throughout the study.

TABLE 8. Mean (± SD) absolute (g) and relative (%) lung weights following inhalation of Zinc Omadine [a]

Time interval/Parameter	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Males				
Day 5				
Terminal body weight (g)	231±8.7	236±9.3	243±14.4	228±15.1
Lungs – Absolute (g)	1.02±0.083	1.19±0.100 (↑17)	1.28±0.124** (↑25)	1.38±0.120** (↑35)
Relative to body wt (%)	0.439±0.0260	0.503±0.0406 (↑15)	0.529±0.0441* (↑21)	0.606±0.0674** (↑38)
Relative to brain wt (%)	54.376±4.8637	62.574±5.5398 (↑15)	68.710±6.7100** (↑26)	74.009±7.8292** (↑36)
Day 12				
Terminal body weight (g)	267±15.6	274±14.0	255±14.0	236±15.4* (↓12)
Lungs – Absolute (g)	1.11±0.092	1.26±0.063 (↑14)	1.33±0.173* (↑20)	1.41±0.147** (↑27)
Relative to body wt (%)	0.418±0.0346	0.459±0.0238 (↑10)	0.521±0.0707* (↑25)	0.596±0.0485** (↑43)
Relative to brain wt (%)	57.641±4.8993	64.799±2.7853 (↑12)	68.063±8.3332* (↑18)	76.137±6.5868** (↑32)
Day 26				
Terminal body weight (g)	327±19.9	319±20.0	303±15.0	274±33.6** (↓16)
Lungs – Absolute (g)	1.29±0.074	1.52±0.140* (↑18)	1.46±0.136 (↑13)	1.55±0.107** (↑20)
Relative to body wt (%)	0.396±0.0274	0.478±0.0522* (↑21)	0.485±0.0510* (↑23)	0.571±0.0429** (↑44)
Relative to brain wt (%)	66.933±3.2310	80.816±8.0284** (↑21)	74.186±7.3403 (↑11)	78.834±4.4502* (↑18)
Females				
Day 5				
Terminal body weight (g)	179±17.8	178±5.9	180±6.7	164±8.6
Lungs – Absolute (g)	0.97±0.075	1.10±0.084 (↑13)	1.22±0.130** (↑26)	1.36±0.113** (↑40)
Relative to body wt (%)	0.544±0.0403	0.616±0.0410 (↑13)	0.680±0.0548** (↑25)	0.833±0.0641** (↑53)
Relative to brain wt (%)	53.468±4.2582	63.424±6.8842 (↑19)	67.169±5.8765* (↑26)	79.942±8.4294** (↑50)
Day 12				
Terminal body weight (g)	185±13.0	186±20.0	189±16.1	177±21.8
Lungs – Absolute (g)	0.97±0.027	1.19±0.159* (↑23)	1.40±0.091** (↑44)	1.40±0.128** (↑44)
Relative to body wt (%)	0.525±0.0386	0.641±0.0857* (↑22)	0.745±0.0510** (↑42)	0.798±0.0573** (↑52)
Relative to brain wt (%)	51.762±0.7167	65.171±6.4714** (↑26)	74.705±4.2397** (↑44)	75.969±5.8364** (↑47)
Day 26				
Terminal body weight (g)	219±12.1	216±17.0	204±18.1	186±36.3
Lungs – Absolute (g)	1.10±0.065	1.30±0.103 (↑18)	1.39±0.127** (↑26)	1.48±0.202** (↑35)
Relative to body wt (%)	0.502±0.0256	0.604±0.0377 (↑20)	0.682±0.0335** (↑36)	0.811±0.1368** (↑62)
Relative to brain wt (%)	58.707±3.1102	72.050±5.6282* (↑23)	73.548±7.9595** (↑25)	79.300±8.3915** (↑35)

a Data obtained from Tables 28 through 33 on pages 122-166 of the study report. Percent differences from controls are included in parentheses. n = 5 in all groups except for the 0.005 mg/L females where n = 4 on Day 26

* Statistically different from the control group at $p < 0.05$

** Statistically different from the control group at $p < 0.01$

TABLE 9. Mean (± SD) absolute (g) and relative (%) thymus weights following inhalation of Zinc Omadine [a]

Time interval/Parameter	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Males				
Day 5				
Terminal body weight (g)	231±8.7	236±9.3	243±14.4	228±15.1
Thymus – Absolute (g)	0.4282±0.10725	0.4258±0.05268	0.3866±0.10442	0.3201±0.05935 (↓25)
Relative to body wt (%)	0.184±0.0411	0.180±0.0160	0.159±0.0391	0.140±0.0193 (↓24)
Relative to brain wt (%)	22.894±5.6145	22.455±2.7168	20.675±5.3371	17.215±3.2807 (↓25)
Day 12				
Terminal body weight (g)	267±15.6	274±14.0	255±14.0	236±15.4* (↓12)
Thymus – Absolute (g)	0.4559±0.07340	0.4041±0.07782	0.4090±0.04170	0.2802±0.04062** (↓39)
Relative to body wt (%)	0.170±0.0215	0.147±0.0226	0.161±0.0173	0.118±0.0132** (↓31)
Relative to brain wt (%)	23.592±3.8463	20.802±3.6827	20.967±1.5353	15.370±1.5980** (↓35)
Day 26				
Terminal body weight (g)	327±19.9	319±20.0	303±15.0	274±33.6** (↓16)
Thymus – Absolute (g)	0.4331±0.10614	0.3917±0.05743	0.3646±0.05954	0.2750±0.09142* (↓37)
Relative to body wt (%)	0.132±0.0249	0.123±0.0201	0.121±0.0209	0.099±0.0256 (↓25)
Relative to brain wt (%)	22.449±5.5262	20.981±4.7315	18.562±3.7462	13.976±4.7877 (↓38)
Females				
Day 5				
Terminal body weight (g)	179±17.8	178±5.9	180±6.7	164±8.6
Thymus – Absolute (g)	0.4203±0.11190	0.4027±0.10311	0.4380±0.13789	0.2782±0.03061 (↓34)
Relative to body wt (%)	0.232±0.0442	0.226±0.0555	0.243±0.0754	0.171±0.0252 (↓26)
Relative to brain wt (%)	23.039±5.5347	23.218±5.9709	23.999±7.2748	16.320±2.0139 (↓29)
Day 12				
Terminal body weight (g)	185±13.0	186±20.0	189±16.1	177±21.8
Thymus – Absolute (g)	0.3806±0.02785	0.3700±0.08263	0.4042±0.04195	0.2616±0.06295* (↓31)
Relative to body wt (%)	0.207±0.0161	0.198±0.0371	0.215±0.0209	0.148±0.0294** (↓29)
Relative to brain wt (%)	20.398±1.5219	20.232±3.8078	21.528±2.0125	14.157±3.2550** (↓31)
Day 26				
Terminal body weight (g)	219±12.1	216±17.0	204±18.1	186±36.3
Thymus – Absolute (g)	0.4005±0.10552	0.3795±0.07637	0.3536±0.11429	0.2393±0.13992 (↓40)
Relative to body wt (%)	0.181±0.0392	0.174±0.0219	0.171±0.0429	0.121±0.0626 (↓33)
Relative to brain wt (%)	21.310±5.3089	20.926±3.9202	18.685±6.1254	12.783±7.5078 (↓40)

a Data obtained from Tables 28 through 33 on pages 122-169 of the study report. Percent differences from controls are included in parentheses. n = 5 in all groups except for the 0.005 mg/L females where n = 5 on Day 5, n = 4 on Day 12, and n = 3 on Day 26.

* Statistically different from the control group at $p < 0.05$

** Statistically different from the control group at $p < 0.01$

3. **Gross pathology:** At 0.005 mg/L, dark red areas in the lungs were observed in the female that died and in the females terminated on schedule on Days 5 (1/5) and 26 (1/4). Dark red areas in the lungs were also noted in one male at 0.0015 mg/L on Day 5. Enlarged bronchial lymph nodes were found in the 0.005 mg/L females on Day 5 (2/5) and Day 12 (1/5) compared to 0 controls. Enlarged mediastinal lymph nodes were found in the 0.0015 mg/L males on Day 12 (1/5), in the 0.0015 mg/L females on Day 26 (1/5), and in the 0.005 mg/L females on Day 26 (1/5). There were no other macroscopic findings which could be attributed to treatment.

4. **Microscopic pathology:** Treatment-related microscopic findings in the lungs were observed in all groups exposed to the test material compared to no findings in the controls (Table 10). In the lungs, minimal to moderate subacute inflammation was observed at 0.0005 mg/L (1/15 males; 5/15 females), 0.0015 mg/L (8/15 males; 13/15 females), and 0.005 mg/L (3/15 males; 7/14 females). Minimal to moderate alveolar macrophages were observed at 0.0005 mg/L (2/15 males; 3/15 females), 0.0015 mg/L (11/15 males; 1/15 females), and 0.005 mg/L (15/15 males; 12/14 females). Minimal to moderate perivasculitis was found at 0.0005 mg/L (11/15 males; 14/15 females), 0.0015 mg/L (13/15 males; 15/15 females), and 0.005 mg/L (15/15 males; 14/14 females). Minimal to moderate hypertrophy of the smooth muscle of the alveolar ducts of the lungs was noted at 0.0005 mg/L (4/14 males; 6/13 females), 0.0015 mg/L (6/13 males; 7/15 females), and 0.005 mg/L (13/15 males; 13/14 females). These findings were considered to be due to direct effects of the test material on the respiratory tract.

   Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to mild degeneration (2/14 treated vs 0/15 controls). There were no other treatment-related findings in the organs and tissues examined microscopically.

TABLE 10. Incidence (# affected) of selected microscopic findings at scheduled terminations [a]

Microscopic finding		Analytical concentration (mg/L)							
		0	0.0005	0.0015	0.005	0	0.0005	0.0015	0.005
		Males				Females			
Lungs (number examined)		15	15	15	15	15	15	15	14
Inflammation, subacute –	Total	0	1	8	3	0	5	13	7
	Minimal	---	1	2	0	---	0	3	0
	Mild	---	---	5	3	---	4	10	6
	Moderate	---	---	1	0	---	1	0	1
Alveolar macrophages –	Total	0	2	11	15	0	3	1	12
	Minimal	---	1	0	0	---	1	0	0
	Mild	---	1	10	15	---	2	1	10
	Moderate	---	0	1	0	---	0	0	2
Perivasculitis –	Total	0	11	13	15	0	14	15	14
	Minimal	---	4	2	0	---	1	0	0
	Mild	---	6	7	10	---	10	9	3
	Moderate	---	1	4	5	---	3	6	11
Smooth muscle hypertrophy (# examined)		15	14	13	15	15	13	15	14
	Total	0	4	6	13	0	6	7	13
	Minimal	---	4	5	6	---	6	7	7
	Mild	---	0	1	5	---	0	0	4
	Moderate	---	0	0	2	---	0	0	2
Skeletal muscle (number examined)		15	15	15	15	15	15	15	14
Degeneration –	Total	0	0	0	0	0	0	0	2
	Minimal	---	---	---	---	---	---	---	1
	Mild	---	---	---	---	---	---	---	1

a Data obtained from Text Table 5 on page 45 of the study report.
--- Severity incidence not applicable because total incidence was zero.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the LOAEL was 0.0005 mg/L based on the local (portal of entry) effects on BALF parameters, lung weights, and microscopic findings in the lungs (i.e., broncho-interstitial pneumonitis and smooth muscle hypertrophy) that were observed at exposure levels of 0.0005 mg/L. The LOAEL for systemic toxicity was 0.005 mg/L based on mortality of one female, clinical signs of toxicity, decreased body weights and food consumption, and degeneration of the skeletal muscle.

B. REVIEWER COMMENTS: At 0.005 mg/L, one female was found dead on Day 15. Histopathology findings consisted of moderate congestion in the lungs, minimal increased mucous in the respiratory epithelium, and mild perivascular inflammation, as well as mild degeneration of skeletal muscle. The findings in the lungs were similar in degree and nature

to the findings in other treated animals. The cause of death was not definitively determined. It was stated that the microscopic findings may have contributed to the death of this animal, but were not the primary cause. Clinical signs of toxicity observed in this animal prior to its death included thinness, dermal atonia, and body weight loss. In the surviving females in this group, treatment-related clinical signs of toxicity included thin body condition in three females and impaired use of the hindlimbs in one female on Day 24 at the 1 hour post-exposure examination. One of these females lost 68 grams during Days 18-25 and was noted as hypothermic on Day 26.

In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased throughout the study compared to controls. A body weight loss was observed for Days 0-4 at this concentration (-6 g) compared to a body weight gain in the controls (13 g). Body weight gain for Days 4-11 was decreased ($p \leq 0.01$), and cumulative body weight gains were decreased ($p \leq 0.01$) in the 0.005 mg/L males for each of the remaining study intervals.

In the 0.005 mg/L females, body weights were decreased (NS) throughout the study. Cumulative body weight gains in the 0.005 mg/L females were lower ($p \leq 0.05$) than controls for each of the intervals throughout the study, and a body weight loss was noted for Days 18-25 (-14 g) compared to a weight gain in the controls (14 g).

Food consumption was dose-dependently decreased ($p \leq 0.05$) in both sexes for Days 0-4 and additionally in the males for Days 4-11.

Treatment-related effects on BALF parameters were noted in all treated groups in both sexes. These effects included increased LDH and total protein and higher percentages of eosinophils, neutrophils, and lymphocytes, and a lower percentage of alveolar macrophages. Mean LDH and total protein levels were higher than control means for all exposure levels on Days 5, 12, and 26, with concentration-related trends observed in the females but not the males. For LDH, intra-group variability was high and statistical significance was limited to the 0.005 mg/L females on Day 5. For total protein, statistical significance was attained in the 0.0015 females (Day 26) and in the 0.005 mg/L males (Days 5 and 26) and females (Days 5, 12, and 26).

For bronchoalveolar lavage cytology, the proportion of eosinophils was increased over controls in a concentration-dependent manner in both sexes at all treatment levels. With the exception of the 0.0005 mg/L males on Day 12, the percentages of eosinophils were highest on Day 5, with progressively lower counts on Days 12 and 26. The higher percentage of eosinophils was more pronounced in females at all exposure levels and at all intervals when compared to males. The proportions of neutrophils and lymphocytes in all treated groups were minimally higher than controls in both sexes. Associated with these changes, the proportions of alveolar macrophages were lower in all treated groups. With the exception of the females on Day 26, the decreases in the percentage of alveolar macrophages were concentration-dependent.

Lung weights were increased in all treated groups in both sexes throughout the study compared to controls. With the single exception of the lung weights relative to brain weight on Day 26 in the males, these increases were concentration-dependent. Statistical significance ($p \leq 0.05$) was attained at 0.0005 mg/L in the males on Day 26 and in the females on Days 12 and 26. The increases at 0.0015 and 0.005 mg/L were statistically significant ($p \leq 0.05$) in males and females at each of the intervals, with the exception of the lung weight relative to brain weight in the 0.0015 mg/L males on Day 26.

Thymus weights were decreased at 0.005 mg/L compared to controls in both sexes throughout the study. The investigators stated that decreases were most likely secondary effects related to stress.

At 0.005 mg/L, dark red areas in the lungs were observed in the female that died and in the females terminated on schedule on Days 5 (1/5) and 26 (1/4). Dark red areas in the lungs were also noted in one male at 0.0015 mg/L on Day 5. Enlarged bronchial lymph nodes were found in the 0.005 mg/L females on Day 5 (2/5) and Day 12 (1/5) compared to 0 controls. Enlarged mediastinal lymph nodes were found in the 0.0015 mg/L males on Day 12 (1/5), in the 0.0015 mg/L females on Day 26 (1/5), and in the 0.005 mg/L females on Day 26 (1/5).

Treatment-related microscopic findings in the lungs were observed in all groups exposed to the test material compared to no findings in the controls. In the lungs, minimal to moderate subacute inflammation was observed at 0.0005 mg/L (1/15 males; 5/15 females), 0.0015 mg/L (8/15 males; 13/15 females), and 0.005 mg/L (3/15 males; 7/14 females). Minimal to moderate alveolar macrophages were observed at 0.0005 mg/L (2/15 males; 3/15 females), 0.0015 mg/L (11/15 males; 1/15 females), and 0.005 mg/L (15/15 males; 12/14 females). Minimal to moderate perivasculitis was found at 0.0005 mg/L (11/15 males; 14/15 females), 0.0015 mg/L (13/15 males; 15/15 females), and 0.005 mg/L (15/15 males; 14/14 females). Minimal to moderate hypertrophy of the smooth muscle of the alveolar ducts of the lungs was noted at 0.0005 mg/L (4/14 males; 6/13 females), 0.0015 mg/L (6/13 males; 7/15 females), and 0.005 mg/L (13/15 males; 13/14 females). These findings were considered to be due to direct effects of the test material on the respiratory tract.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to mild degeneration (2/14 treated vs 0/15 controls). There were no other treatment-related findings in the organs and tissues examined microscopically.

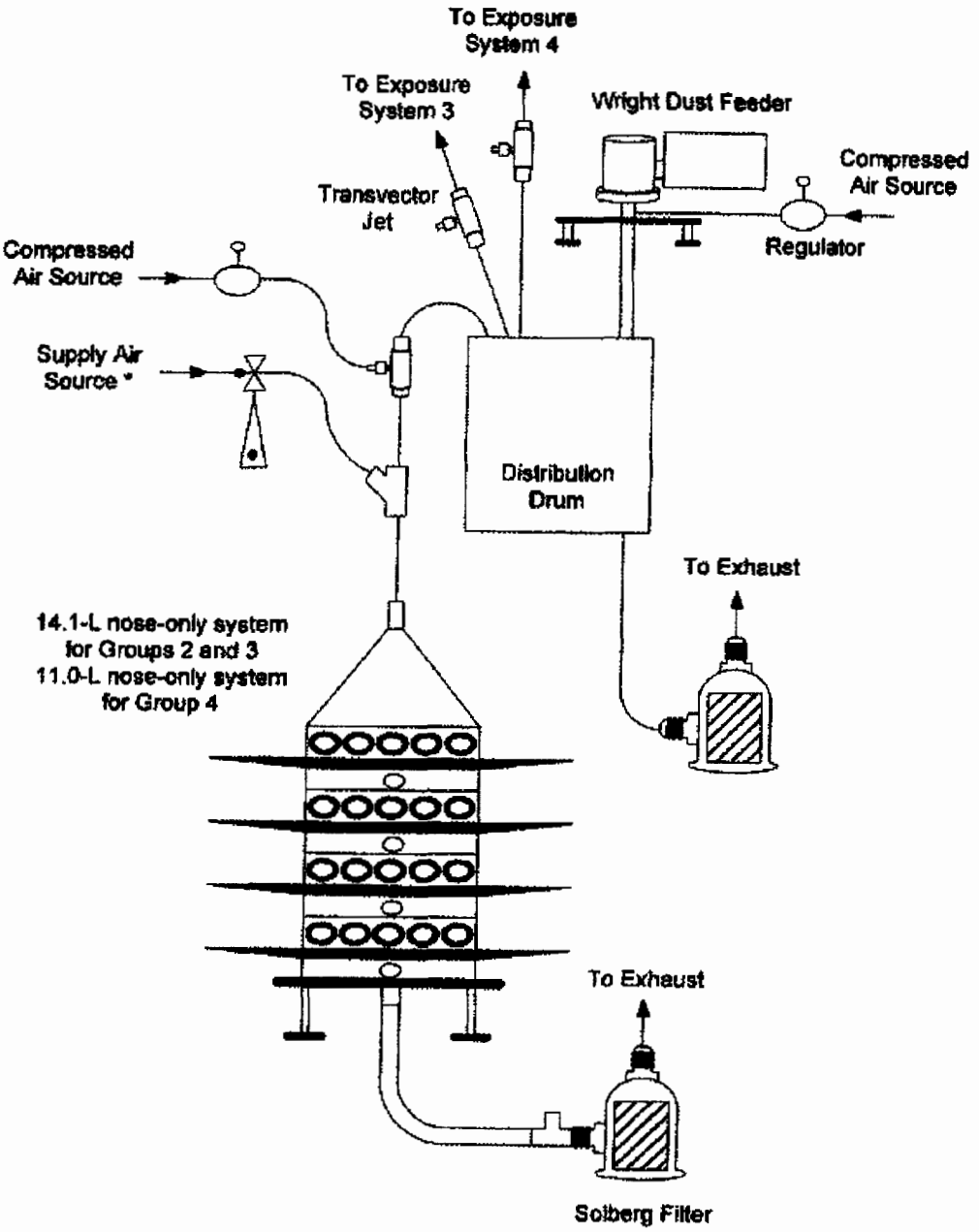
The LOAEL is 0.0005 mg/L based on increases in BALF parameters (increased LDH, total protein, and percentages of neutrophils and lymphocytes), lung weights, and incidences of microscopic findings in the lungs (subacute inflammation, alveolar macrophages, perivasculitis, and hypertrophy of the smooth muscle of the alveolar ducts). A NOAEL was not established.

This 28-day study is classified as **acceptable/non-guideline.** This study was conducted after

review of a protocol submitted by the registrant as part of discussions between the Antimicrobials Division and the registrant to determine toxic similarity between zinc pyrithione and copper pyrithione. The purpose of this study with zinc pyrithione was to examine toxic effects of zinc pyrithione by inhalation after 4 weeks, which included examination of lung bronchioalveolar lavage fluid after single or repeated inhalation exposures, and examination of lung histopathology. Certain parameters (microscopic examination of nasal passages, trachea, and larynx; neurobehavioral, ophthalmologic, and clinical pathology examinations) were not conducted in this study. A similar study has been conducted with copper pyrithione.

APPENDIX

FIGURE 1: ATMOSPHERE GENERATION AND EXPOSURE SYSTEM



* On 23 July 2009, the supply air was split and delivered to 2 positions along the delivery line following the transvector.

EPA Reviewer: Jonathan Chen, Ph.D. **Signature:** Jonathan Chen
RASSB, Antimicrobial Division **Date:** 02/02/2011
Work Assignment Manager: Steve Malish, Ph.D. **Signature:** S.J. Malish
RASSB, Antimicrobial Division **Date:** 2/7/11

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose 28-Day Oral Toxicity Study in Rats; OPPTS 870.3050; OECD 407.

PC CODE: 088001 **DP BARCODE:** 369393
TEST MATERIAL (PURITY): Copper pyrithione (99.7% a.i.)

SYNONYMS: Copper-2-pyrithio-1-oxide, Copper Omadine

CITATION: Omori, M. (1995) A repeated dose toxicity study of copper pyrithione administered orally to rats for 28 days followed by a 14-day recovery period. Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. Laboratory Project ID: SBL 40-46, July 18, 1995. MRID 45774311. Unpublished.

SPONSOR/SUBMITTER: Arch Chemicals Inc., 501 Merritt 7, Norwalk, CT

EXECUTIVE SUMMARY: In a repeated dose, oral toxicity study (MRID 45774311), copper pyrithione (99.7% a.i.; Batch No. 9302095981) in 0.5% (w/v) carboxymethylcellulose was administered daily via gavage (10 mL/kg) to Sprague-Dawley rats at dose levels of 0, 0.6, 2.5, or 10 mg/kg/day for 28 days; the control and high-dose groups used 10 rats/sex/group and the low and mid-doses used 5 rats/sex/group. For the 0 and 10 mg/kg/day groups, 5 rats/sex/group were retained for a 14-day recovery period.

At 10 mg/kg/day, two females were sacrificed *in extremis* on Day 17 of the dosing period. Additionally, one 10 mg/kg/day female was found dead on Day 2 of the recovery period. These three animals exhibited the following clinical signs prior to death or euthanasia: emaciation; decreased spontaneous activity, piloerection, ataxic gait, paralysis of the hind leg, urine-stained abdomen, reddish eye gum; prone position; and lateral position. Additionally, hypothermia and bradypnea were noted in the females sacrificed in moribund condition, and a trace of reddish rhinorrhea was observed in the animal found dead. Among the surviving 10 mg/kg/day females, similar clinical signs of emaciation, piloerection, decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg; reddish eye gum; urine-stained abdomen; and prone position were found. However, almost all of these findings decreased in severity or occurrence during the final week of dosing. During the initial recovery period, decreased spontaneous activity, ataxic gait, and piloerection were still observed in 1 or 2 females, but these abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females during the recovery period, but these animals appeared to be recovering.

No treatment-related clinical signs were noted in the 10 mg/kg/day males, other than slight emaciation in three males on Days 21 to 28 or on Days 0-2 of the recovery period,

Body weights were decreased ($p<0.05$) by 10-13% in the 10 mg/kg/day males during Weeks 3 and 4 and by 19-36% in the females during Weeks 2-4. During the recovery period, body weights remained decreased by 13% in these males during Week 1 and by 25-33% in the females during Weeks 1 and 2. Body weight gains were decreased ($p<0.05$) by 18-39% in the males during Weeks 2-4. The females at this dose actually lost significant ($p<0.01$) weight during Week 2 (-0.6 g) and Week 3 (-18.8 g) compared to gains of 24.6 and 28.6 g, respectively, in the controls. Overall (Weeks 0-4) body weight gain was decreased by 22% in the males and 76% in the females. During the recovery period, body weight gains were increased compared to controls by 28% in the males during Week 2 and by 82-123% in females during Weeks 1 and 2. Food consumption was decreased ($p<0.01$) by 11-12% in the males during Weeks 3 and 4 and by 34-37% in the females during Weeks 2 and 3. Food consumption was similar to controls in both sexes during the recovery period.

At the high-dose, treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period. Microscopic observations of muscle fiber atrophy were observed in the gastrocnemius, soleus, flexor digitorum longus, and anterior tibial muscles in all (5/5) females at this dose compared to 0/5 controls. Additionally, muscle fiber atrophy of the biceps femoris was found in 2/5 females at 10 mg/kg/day compared to 0/5 controls. In the high-dose males, muscle fiber atrophy in the anterior tibial muscle was observed in 2/5 rats compared to 0/5 controls. The severity of the muscle fiber atrophy was generally very slight to slight.

No treatment-related effects were observed at 0.6 or 2.5 mg/kg/day in either sex.

The LOAEL was 10 mg/kg/day based on decreased body weight, body weight gain, and food consumption in both sexes and on mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. The NOAEL is 2.5 mg/kg/day.

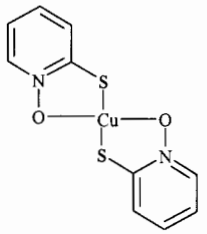
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Copper pyrithione

Description:	Olive-green powder
Batch #:	9302095981
Purity:	99.7% a.i.
Stability:	The test material was shown to be stable in the vehicle for up to 24 hrs at room temperature.
CAS # of TGAI:	14915-37-8
Structure:	

2. Vehicle: Aqueous 0.5% (w/v) carboxymethylcellulose

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley, SPF Crj:CD(SD)
Age/ weight at study initiation:	Approximately 5 weeks/ 143-161 g males and 121-134 g females
Source:	Charles River Japan, Inc.
Housing:	Individually in stainless steel cages
Diet:	CE-2 solid food (sterilized by cobalt-60 irradiation ; CLEA Japan, Inc.), *ad libitum*; except during fasting prior to blood collection
Water:	Tap water, *ad libitum*
Environmental conditions	
Temperature:	22±2°C
Humidity:	50±10%
Air changes:	15/hr
Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	8 days

B. STUDY DESIGN

1. In-life dates: Not reported

2. Animal assignment: The animals were randomly assigned, stratified by weight, to the test groups presented in Table 1.

TABLE 1. Study design [a]			
Test group	Dose (mg/kg/day)	# Males [b]	# Females [b]
Control	0	5+5	5+5
Low	0.6	5	5
Mid	2.5	5	5
High	10	5+5	5+5

[a] Data were extracted from page 15 of the study report.

[b] An additional 5 rats/sex were added to the control and high-dose groups for the recovery test.

3. **Dose-selection rationale:** The doses in the current study were based on the results of a preliminary repeated dose toxicity study (Report No. SBL 40-45; Omori, M., 1995), in which rats were exposed orally to copper pyrithione at 1, 5, and 25 mg/kg/day for 2 weeks. It was determined that 25 mg/kg/day caused significant toxicity but no mortality, and 5 mg/kg/day caused no significant toxicity. Therefore, 10 mg/kg/day was selected as the high-dose in the current study.

4. **Treatment preparation, administration, and analysis:** Dose formulations were prepared daily by mixing the appropriate amounts of copper pyrithione and 0.5% (w/v) carboxymethylcellulose. The animals were dosed daily via gavage at a volume of 10 mL/kg based on the most recent body weight. Stability after 24 hrs at room temperature and homogeneity (top, middle, bottom) were evaluated in 0.06, 0.10, and 2.5 mg/mL formulations prior to the study. Concentration analyses were performed using samples from each dose formulation on the initial day of dosing and at Week 4 during the current study.

Results

Homogeneity analysis (%CV): 0-1%

Stability analysis (% of initial value): 94.9-101.7% (after 24 hrs at room temperature)

Concentration analysis (range as mean % of nominal):

Concentration (mg/mL)	Mean % of nominal
0.06	95-100
0.25	97.6-104
1.0	100-108

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The following statistical methods were applied to the data, and significance was denoted at $p<0.05$ or $p<0.01$. Body weight, food consumption, hematology, clinical chemistry, and organ weight data were first analyzed for homogeneity of variance by Bartlett's test. If homogeneity of variance existed, a one-way ANOVA was applied. If the ANOVA was significant, Dunnett's test (equal numbers of data) or Scheffé's test (unequal

number of data) was used to compare the treatment and control groups. If the variance was heterogeneous in Bartlett's test, the order was converted and the Kruskal-Wallis H test was applied. If the results were significant, Dunnett-type test (equal numbers of data) or Scheffe-type test (unequal numbers of data) was used to compare the mean ranks. Urinalysis, macroscopic pathology, and microscopic pathology data were analyzed using either the exact rank sum test (gradable values) or the Fisher's exact test (non-gradable values). Clinical signs data were not subjected to statistical analysis.

C. METHODS

1. Observations

a. Cageside observations: Animals were observed for mortality and clinical signs of toxicity three times daily (prior to dosing, and at approximately 1-2 hr and 4-5 hr post-dosing) during the dosing period, and once daily during the recovery period.

b. Clinical examinations: It was not reported if the animals were subjected to detailed physical examinations.

c. Neurological evaluations: All animals were observed for general behavior according to the Irwin method[1] (a modified FOB) once prior to initiation of dosing and on Days 0, 6, 13, 20, and 27 of the dosing period and on Day 6 and 13 of the recovery period.

The parameters examined included, but were not limited to, the following: awareness (alertness, visual placing, stereotypy, and passivity); mood (grooming and vocalization); motor activity (reactivity, spontaneous activity, touch response, and pain response); central nervous system excitation (startle response, straub tail, tremors, convulsions, and twitches); posture; motor incoordination (staggering gait, abnormal gait, and righting reflex); muscle tone (grip strength, body tone, and abnormal tone); reflex (pinna reflex, corneal reflex, and ipsilateral flexor reflex); and autonomic profile (writhing, palpebral opening, exophthlmos, urination, salivation, piloerection, hypothermia, skin color, heart rate, and respiratory rate. Details of the scoring scale were not provided in the study report.

2. Body weight: All animals were weighed once prior to initiation of dosing and weekly thereafter. Body weight gains were also calculated weekly.

3. Food consumption: Food consumption (g/animal/day) was calculated once prior to initiation of dosing and weekly thereafter.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed.

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1 Irwin S., Animal and clinical pharmacological techniques in drug evaluation. Ed. By Nodine, J.H. and Siegler, P.E., Chicago, Year Book Medical Publishers, 36-54, 1964.

5. **Hematology and clinical chemistry:** On the day of scheduled sacrifice, all surviving animals (fasted for 16-24 hr) were anesthetized by injection of sodium pentobarbital. Blood samples for hematology parameters were collected from the abdominal vein and treated with EDTA-2K or 3.8% sodium citrate (clotting measurements). Afterward, blood samples for clinical chemistry evaluations were collected from the abdominal aorta. The following CHECKED (X) parameters were examined:

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Kaolin-cephalin time)		
X	(Prothrombin time)		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)	X	Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein*
X	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

6. **Urinalysis:** Urine was collected using the compulsory method once prior to initiation of dosing, once during Week 4 (prior to daily dosing) of the dosing period, and once during Week 2 of the recovery period. Color was judged visually and the following parameters were determined using Multistix® test paper (Miles-Sankyo Co., Inc.).

X	Appearance*	X	Glucose
	Volume*	X	Ketones
	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Optional for 28-day oral rodent studies

7. **Sacrifice and pathology:** All animals sacrificed *in extremis* and at scheduled necropsy were anesthetized by injection of sodium pentobarbital and euthanized after blood collection. The following CHECKED (X) tissues were collected, and the (XX) organs were also weighed (paired organs were weighed separately and together).

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*+
	Salivary glands*	X	Heart*+	X	Peripheral nerve*
	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
	Stomach*		Lymph nodes*	X	Pituitary*
	Duodenum*	XX	Spleen*+		Eyes (w/optic nerve)*
	Jejunum*		Thymus*+		**GLANDULAR**
	Ileum*			XX	Adrenal glands*+
	Cecum*		**UROGENITAL**		Lacrimal glands
	Colon*	XX	Kidneys*+	X	Parathyroids*
	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*		Epididymides*+		Bone (sternum and femur)
	Bile duct (rat)		Prostate*	X	Skeletal muscle
	Pancreas*		Seminal vesicles*		Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
	Trachea*		Uterus*+		
XX	Lungs*		Mammary gland*		
	Nose*		Cervix		
	Pharynx*		Vagina		
	Larynx*				

* Recommended for 28-day oral rodent studies based on Guideline 870.3050
\+ Organ weights required for rodent studies.

The eyes were fixed in a mixture of formaldehyde and glutaraldehyde. The remaining collected tissues were fixed in 10% neutral buffered formalin. Tissues from 5 rats/sex/dose in the control and 10 mg/kg/day groups and the triceps muscle of the calf (gastrocnemius

muscle and soleus muscle), flexor digitorum longus muscle, anterior tibial muscle, liver, kidneys, and gross lesions from all groups were evaluated microscopically. Tissues were routinely processed, embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** At 10 mg/kg/day, two females (nos. 53 and 54) were sacrificed *in extremis* on Day 17 of the dosing period. Additionally, one 10 mg/kg/day female (no. 51) was found dead on Day 2 of the recovery period. All these deaths are treatment related.

2. **Clinical signs:** At 10 mg/kg/day, two females (nos. 53 and 54) were sacrificed in extremis on Day 17 of the dosing period. These animals displayed the following clinical signs: emaciation from Day 14 of the dosing period onward; decreased spontaneous activity, piloerection, ataxic gait, and/or paralysis of the hind leg, urine stained abdomen, and reddish eye gum on Day 15 and thereafter; prone position on Day 16; and hypothermia, bradypnea, and lateral position were noted on Day 17. While under observation using the Irwin method, no abnormalities were noted in either of these animals until Day 13 of the dosing period.

   Additionally, one 10 mg/kg/day female (no. 51) was found dead on Day 2 of the recovery period. This animal displayed the following clinical signs prior to death: emaciation from Day 17 of the dosing period onward; decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg, urine stained abdomen, reddish eye gum, and a trace of reddish rhinorrhea from Day 18 onward; piloerection from Day 19 onward; prone position on Day 0 of the recovery period; and lateral position on Day 1 of the recovery period. While under observation using the Irwin method, an increase in passivity, decreases in reactivity, spontaneous activity, pain response, limb tone and grip strength, and loss of ipsilateral flexor reflex, abnormal hind limb posture and piloerection were observed on Day 20 and 27 of the dosing period. On Day 27, abnormal urination was also observed. All other animals survived to scheduled sacrifice.In the surviving 10 mg/kg/day females, emaciation was observed in all animals on Days 12 and 20 of the dosing period. Thereafter, piloerection decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg were observed in 6 animals; reddish eye gum was noted in 3 animals; urine stained abdomen was noted in 2 animals; and prone position was noted in 1 animal. However, almost all of these findings disappeared or tended to recover by the end of the dosing period. Other than slight emaciation observed in three 10 mg/kg/day males on Days 21 to 28 or during Days 0-2 of the recovery period, no treatment-related clinical signs were noted in the males at any dose.

3. **Neurological evaluations (Irwin method):** No treatment-related differences from controls were noted in the males at any time-point or at any dose. Scores for all parameters were similar to controls prior to initiation of treatment and on Days 0, 6, and 13 of dosing in the

treated females. Incidence (# affected/8 vs. 0/10 controls) of the following observations were noted in the 10 mg/kg/day females on Day 20 (Table 2): (i) increased passivity (7); (ii) decreased reactivity (3); (iii) decreased spontaneous activity (5); (iv) decreased pain response (7); (v) abnormal hind limb posture (7); (vi) staggering gait (7); (vii) abnormal gait (5); (viii) decreased limb tone (7); (ix) decreased grip strength (7); (x) loss or decrease in ipsilateral flexor reflex (7); (xi) abnormal urination (2) and (xii) piloerection (7).. On Day 27, the same FOB parameters still showed an effect of treatment in the 10 mg/kg/day females; however, with the exception of reactivity and piloerection, the incidence and severity were generally less than that observed on Day 20. During the recovery period, a decrease in spontaneous activity and piloerection were observed in 2 females and ataxic gait was noted in 1 female continuously from the dosing period. These abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females but appeared to be recovering.

TABLE 2. Incidence (# affected) of selected FOB parameters in female rats treated with copper pyrithione for up to 28 days. [a]

Observation	Score [b]	Dose (mg/kg/day)							
		0 (n=10)	0.6 (n=5)	2.5 (n=5)	10 (n=8)	0 (n=10)	0.6 (n=5)	2.5 (n=5)	10 (n=8)
		Day 20				Day 27			
Passivity (normal = 0)	0	10	5	5	1	10	5	5	5
	1	0	0	0	7	0	0	0	3
Reactivity (normal = 4)	4	10	5	5	5	10	5	5	4
	3	0	0	0	3	0	0	0	4
Spontaneous activity (normal = 4)	4	10	5	5	3	10	5	5	4
	3	0	0	0	0	0	0	0	2
	2	0	0	0	5	0	0	0	2
Pain response (normal = 4)	4	10	5	5	1	10	5	5	4
	3	0	0	0	2	0	0	0	2
	2	0	0	0	2	0	0	0	1
	1	0	0	0	3	0	0	0	1
Limb posture (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	7	0	0	0	3
Staggering gait (normal = 0)	0	10	5	5	1	10	5	5	5
	2	0	0	0	2	0	0	0	2
	-	0	0	0	5	0	0	0	1
Abnormal gait (normal = 0)	0	10	5	5	3	10	5	5	7
	-	0	0	0	5	0	0	0	1
Limb tone (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	5	0	0	0	2
	2	0	0	0	2	0	0	0	1
Grip strength (normal =4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	7	0	0	0	3
Ipsilateral flexor reflex (normal = 4)	4	10	5	5	1	10	5	5	5
	3	0	0	0	2	0	0	0	0
	2	0	0	0	0	0	0	0	2
	0	0	0	0	5	0	0	0	1
Urination (normal = 0)	0	10	5	5	6	10	5	5	7
	1	0	0	0	1	0	0	0	1
	2	0	0	0	1	0	0	0	0
Piloerection (normal = 0)	0	10	5	5	1	10	5	5	0
	2	0	0	0	7	0	0	0	8

[a] Data were extracted from Tables 2-9 and 2-10 on pages 42 and 43 of the study report.

[b] Irwin method (1964)

B. BODY WEIGHT AND BODY WEIGHT GAIN: At 10 mg/kg/day, body weights were decreased ($p<0.05$) by 10-13% in the males during Weeks 3 and 4 and by 19-36% in the females during Weeks 2-4 (Table 3a). During the recovery period, body weights remained decreased by 13% in the males during Week 1 and by 25-33% in the females during Weeks 1-2. No effects on body weight were observed at 0.6 or 2.5 mg/kg/day in either sex.

TABLE 3a. Mean (±SD) body weights (g) in rats treated with copper pyrithione for up to 28 days. [a]

Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Dosing period				
Males				
0	151.7±4.6	152.6±5.9	152.6±4.4	150.6±4.3
3	323.0±20.7	323.6±12.0	315.6±20.5	291.2±19.7* (↓10)
4	363.1±27.8	364.8±21.5	348.4±26.1	315.8±25.0** (↓13)
Females				
0	127.2±2.8	128.4±2.7	128.4±3.6	127.1±3.7
2	190.5±11.7	188.8±6.0	190.2±7.6	154.6±20.7** (↓19)
3	215.0±17.2	208.6±10.5	209.2±9.9	137.5±22.4** (↓36)
4	237.9±21.6	227.6±18.8	228.2±5.7	153.6±24.8** (↓35)
Recovery period				
Males				
1	398.6±38.5	--	--	347.0±31.3* (↓13)
2	428.2±41.1	--	--	384.8±33.5
Females				
1	245.4±23.4	--	--	164.5 (↓33)
2	258.2±21.4	--	--	193.0 (↓25)

[a] Data were extracted from Tables 4-1 and 4-2 on pages 46 and 47 of the study report. Percent difference from control is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

Body weight gains were decreased ($p<0.05$) by 18-39% in the 10 mg/kg/day males during Weeks 2-4 (Table 3b). The females at this dose actually lost significant ($p<0.01$) weight during Week 2 (-0.6 g) and Week 3 (-18.8 g) compared to 24.6 and 28.6 g, respectively, in the controls. At 10 mg/kg/day, overall (Weeks 0-4) body weight gain was decreased by 22% in the males and 76% in the females. During the recovery period, body weight gains were increased compared to controls by 28% in the males during Week 2 and by 82 and 123% in females during Weeks 1 and 2, respectively.

TABLE 3b. Mean (±SD) body weight gains (g) in rats treated with copper pyrithione for up to 28 days. [a]				
Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Dosing period				
Males				
1	59.0±7.1	60.8±5.6	58.4±7.8	55.8±5.8
2	62.0±6.1	62.0±3.3	58.8±4.0	51.0±8.8* (↓18)
4	40.1±10.9	41.2±10.3	32.8±9.2	24.6±8.0* (↓39)
Overall (0-4) gain [b]	211.4	212.2	195.8	165.2 (↓22)
Females				
1	34.7±7.8	32.6±3.6	35.8±4.1	28.1±5.7
2	28.6±4.6	27.8±5.5	26.0±3.6	-0.6±16.5**
3	24.5±7.2	19.8±6.3	19.0±2.7	-18.8±23.5**
4	22.9±6.2	19.0±9.1	19.0±5.5	16.1±16.5
Overall (0-4) gain [b]	110.7	99.2	99.8	26.5 (↓76)
Recovery period				
Males				
1	29.0±7.4	--	--	30.8±6.7
2	29.6±5.3	--	--	37.8±5.7* (↑28)
Females				
1	14.8±7.5	--	--	27.0 (↑82)
2	12.8±2.4	--	--	28.5 (↑123)

[a] Data were extracted from Tables 4-3 and 4-4 on pages 48 and 49 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

[b] Calculated by reviewers from data within Table 3a above.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

C. **FOOD CONSUMPTION:** At 10 mg/kg/day, food consumption was decreased ($p<0.01$) by 11-12% in the males during Weeks 3 and 4 and by 34-37% in the females during Weeks 2 and 3 (Table 4). Food consumption was similar to controls in both sexes during the recovery period. No effects on food consumption were observed at 0.6 or 2.5 mg/kg/day in either sex.

TABLE 4. Mean (±SD) food consumption (g/animal/day) in rats treated with copper pyrithione for up to 28 days. [a]				
Interval (Weeks)	Dose (mg/kg/day)			
	0	0.6	2.5	10
Males				
0	21.6±1.3	21.6±1.3	21.4±1.3	20.9±1.2
3	28.2±2.5	28.6±1.7	26.8±1.9	24.7±1.8** (↓12)
4	28.7±2.2	27.4±3.5	24.8±2.6	24.3±2.5** (↓11)
Females				
0	18.7±0.7	17.8±1.6	19.0±1.9	18.5±1.6
2	20.0±1.8	19.6±1.5	20.0±2.0	13.2±4.4** (↓34)
3	20.8±1.7	19.2±1.3	19.6±2.3	13.1±5.6** (↓37)
4	20.8±3.0	19.0±2.7	19.8±1.5	16.6±6.1

[a] Data were extracted from Tables 3-1 and 3-2 on pages 44 and 45 of the study report. Percent difference from control (calculated by reviewers) is presented parenthetically.

** Significantly different from controls at $p<0.01$

D. OPHTHALMOSCOPIC EXAMINATION: Not performed.

E. BLOOD ANALYSES

1. **Hematology:** At 10 mg/kg/day, treatment-related differences ($p<0.05$) in hematology parameters were limited to decreased lymphocyte count in the males (↓31%) and increased segmented neutrophilic ratio in the females (6.8% treated vs. 2.8% controls). The increases in platelets (↑54%) and reticulocytes (↑50%) noted in the 10 mg/kg/day females at the end of the recovery period were considered incidental as these parameters were unaffected during dosing. All other statistically significant differences in hematology parameters were unrelated to dose.

2. **Clinical chemistry:** At 10 mg/kg/day, decreased ($p<0.05$) ALT (↓35%) and alkaline phosphatase (↓21%) were noted in the males at the end of the dosing period. Because these levels were decreased, as opposed to increased, the differences were not considered adverse. At the end of the recovery period, the following differences ($p<0.05$) were noted in the 10 mg/kg/day group: decreased triglyceride value in both sexes (↓31-57%); increased cholinesterase activity in the males (↑20%); elevated A/G ratio in the females (↑16%); and elevated chloride levels in the females (↑3%). Although the data were not provided, it was stated that the individual values for all of the above parameters were within the range of historical controls (control background data, 1995). With the exception of the significantly decreased ($p<0.05$) ALT and alkaline phosphatase, none of the above-mentioned differences were observed during the treatment period; therefore, these findings are considered incidental. All other statistically significant differences noted in clinical chemistry parameters were minor and/or unrelated to dose.

F. URINALYSIS: No treatment-related effects on urinalysis parameters were noted at any dose in either sex.

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** No treatment-related differences from controls were observed in absolute organ weights at any dose in either sex during the dosing or recovery periods. Relative (to body) kidney weight was increased ($p<0.05$) by 22% in the 10 mg/kg/day males at the end of the dosing period. Additionally, the following increases ($p<0.05$) in relative (to body) organ weights were observed in the 10 mg/kg/day females at the end of the dosing period: (i) kidney (↑28%); (ii) adrenals (↑32%); (iii) spleen (↑39%); (iv) brain (↑43%); (v) lungs (↑34%); and (vi) liver (↑43%). However, as no corroborative histopathological findings were observed, these decreases were attributed to the decreased body weights observed at this dose.

2. **Gross pathology:** At 10 mg/kg/day, treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period.

3. **Microscopic pathology:** In the 10 mg/kg/day females, increased incidence (# affected/5 vs. 0/5 controls) of muscle fiber atrophy was observed in the following tissues at the end of the dosing period (Table 5): (i) biceps femoris (2, slight); (ii) gastrocnemius (5, very slight to moderate); (iii) soleus muscle (5, very slight to slight); (iv) flexor digitorum longus (5, very slight to slight); and (v) anterior tibial muscle (5, very slight to slight). Very slight muscle fiber atrophy in the anterior tibial muscle was also observed in 2/5 males at 10 mg/kg/day (vs. 0/5 controls). All other microscopic differences from controls were considered incidental as they were commonly observed in rats, the severity was similar to controls, and/or they were unrelated to dose.

   At the end of the recovery period, none of the males had any microscopic lesions. Very slight to slight muscle fiber atrophy was found in 2/5 females at 10 mg/kg/day in the gastrocnemius soleus muscle, flexor digitorum longus and anterior tibial muscle.

TABLE 5. Incidence (# affected/5) of muscle fiber atrophy in rats treated with copper pyrithione for 28 days. [a]

Observation		Dose (mg/kg/day)							
		0	0.6	2.5	10	0	0.6	2.5	10
		Males				Females			
Biceps femoris	Slight	0	0	0	0	0	0	0	2
Gastrocnemius	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	3
	Moderate	0	0	0	0	0	0	0	1
Soleus muscle	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	0	0	0	0	0	4
Flexor digitorum longus muscle	Total	0	0	0	0	0	0	0	5
	Very slight	0	0	0	0	0	0	0	2
	Slight	0	0	0	0	0	0	0	3
Anterior tibial muscle	Total	0	0	0	2	0	0	0	5
	Very slight	0	0	0	2	0	0	0	2
	Slight	0	0	0	0	0	0	0	3
Recovery period									
Gastrocnemius	Total	0	--	--	0	0	--	--	2
	Very slight	0	--	--	0	0	--	--	1
	Slight	0	--	--	0	0	--	--	1
Soleus muscle	Very slight	0	--	--	0	0	--	--	2
Flexor digitorum longus muscle	Very slight	0	--	--	0	0	--	--	2
Anterior tibial muscle	Very slight	0	--	--	0	0	--	--	2

[a] Data were extracted from Tables 11-2, 11-4, and 11-8 on pages 85, 87, and 91 of the study report.
-- Not evaluated

III. DISCUSSION AND CONCLUSIONS

A. REVIEWER'S COMMENTS: At 10 mg/kg/day, two females were sacrificed *in extremis* on Day 17 of the dosing period. These animals displayed the following clinical signs: emaciation from Day 14 of the dosing period onward; decreased spontaneous activity, piloerection, ataxic gait, and/or paralysis of the hind leg, urine stained abdomen, and reddish eye gum on Day 15 and thereafter; prone position on Day 16; and hypothermia, bradypnea, and lateral position on Day 17. Additionally, one 10 mg/kg/day female was found dead on Day 2 of the recovery period. This animal displayed the following clinical signs prior to death: emaciation from Day 17 of the dosing period onward; decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg, urine stained abdomen, reddish eye gum, and a trace of reddish rhinorrhea from Day 18 onward; piloerection from Day 19 onward; prone position on Day 0 of the recovery period; and lateral position on Day 1 of the recovery period. While under observation using the Irwin method, an increase in passivity, decreases in reactivity, spontaneous activity, pain response, limb tone and grip strength, and loss of ipsilateral flexor reflex, abnormal hind limb posture and piloerection were observed on Days 20 and 27 of the dosing period. On Day 27, abnormal urination was also observed.

In the surviving 10 mg/kg/day females, emaciation was observed in all animals on Days 12 and 20 of the dosing period. Thereafter, piloerection decreased spontaneous activity, ataxic gait and/or paralysis of the hind leg were observed in 6 animals; reddish eye gum was noted in 3 animals; urine stained abdomen was noted in 2 animals; and prone position was noted in 1 animal. However, almost all of these findings disappeared or tended to recover by the end of the dosing period. Other than slight emaciation observed in three males on Days 21 to 28 or during Days 0-2 of the recovery period, no treatment-related clinical signs were noted in the males at any dose. During the FOB, incidence of the following observations were noted in the 10 mg/kg/day females on Day 20: (i) increased passivity; (ii) decreased reactivity; (iii) decreased spontaneous activity; (iv) decreased pain response; (v) abnormal hind limb posture; (vi) staggering gait; (vii) abnormal gait; (viii) decreased limb tone; (ix) decreased grip strength; (x) loss or decrease in ipsilateral flexor reflex; (xi) piloerection; and (xii) abnormal urination. On Day 27, the same FOB parameters still showed an effect of treatment in females at this dose; however, both incidence and severity were generally less than that observed during Day 20 observations. During the recovery period, a decrease in spontaneous activity and piloerection were still observed in 2 females and ataxic gait was noted in 1 female continuously from the dosing period. These abnormalities disappeared by Day 5 of the recovery period. Emaciation was also observed in 2 females but appeared to be recovering. Body weights were decreased in the males during Weeks 3 and 4 and in the females during Weeks 2-4. During the recovery period, body weights remained decreased in the males during Week 1 and in the females during Weeks 1-2. Body weight gains were decreased in the males during Weeks 2-4. The females at this dose actually lost weight during Weeks 2 and 3. Overall (Weeks 0-4) body weight gain was decreased in both sexes. During the recovery period, body weight gains were increased compared to controls in the males during Week 2 and in females during Weeks 1 and 2. Food consumption was decreased in the males during Weeks 3 and 4 and in the females during Weeks 2 and 3. Food

consumption was similar to controls in both sexes during the recovery period. Treatment-related gross lesions were limited to slight to moderate atrophy of the biceps femoris in 2/5 females and slight to marked emaciation in all females at the end of the dosing period. In females at this dose, increased incidence of very slight to moderate muscle fiber atrophy was observed in the following tissues: (i) biceps femoris; (ii) gastrocnemius; (iii) soleus muscle; (iv) flexor digitorum longus; and (v) anterior tibial muscle. Very slight muscle fiber atrophy in the anterior tibial muscle was also observed in the males at this dose (2/5 treated vs. 0/5 controls).

No treatment-related effects were observed at 0.6 or 2.5 mg/kg/day in either sex.

The LOAEL was 10 mg/kg/day based on decreased body weight, body weight gain, and food consumption in both sexes and on mortality, clinical signs of toxicity, and muscle fiber atrophy in the females. The NOAEL is 2.5 mg/kg/day.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3050; OECD 407) for a subchronic oral toxicity study in the rat.

B. STUDY DEFICIENCIES: The following minor deficiencies were noted, but do not affect the conclusions or acceptability of this DER:

- Ophthalmoscopic evaluations were not performed.
- Several tissues were not collected and/or weighed during histopathological examinations.
- Details of the scoring scale for the Irwin method were not provided.
- Historical control data for the cited clinical chemistry data were not provided.

Sign-off Date : 02/15/11
DP Barcode Nos.: D3375749 and D369393

TXR No. : 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D
RASSB, Antimicrobial Division
Work Assignment Manager: Steve Malish, Ph.D
RASSB, Antimicrobial Division

Signature: Jonathan Chen
Date: 02/02/2011
Signature: Steven J. Malish
Date: 2/7/11

DATA EVALUATION RECORD

STUDY TYPE: 9-Day Toxicity and Metabolism Study in Rats (gavage); Non-Guideline

PC CODE: 088001 **DP BARCODE:** 369393

TEST MATERIAL (RADIOCHEMICAL PURITY): Copper pyrithione (>98%)

SYNONYMS: Copper-2-pyrithio-1-oxide, Copper Omadine

CITATION: Valentine, J.L. (2002). Final report – amended: *in vivo* dissociation of copper pyrithione (CuPT) in female rats. Bioorganic Chemistry, RTI International, Research Triangle Park, NC. Laboratory Project ID: 08363.000, August 5, 2002. MRID 45774326. Unpublished.

SPONSOR: Arch Chemical Inc., 501 Merrit 7, Norwalk, CT

EXECUTIVE SUMMARY: In a non-guideline special study (MRID 45774326), female Sprague-Dawley rats were treated with copper or zinc pyrithione (non-radiolabeled chemical purity not reported; >98% radiochemical purity; Batch/Lot # not reported) in aqueous 0.5% Darvan (sodium polynaphthalenesulfonate) by daily oral gavage in a dose volume of 5 mL/kg. An initial range-finding study was performed to investigate the toxicity. In this study, animals (3/dose group) were dosed for 9 consecutive days with vehicle or 4, 6, 9, or 12 mg/kg/day copper pyrithione (CuPT) or 3, 5, 8, or 11 mg/kg/day zinc pyrithione (ZnPT). Mortality, clinical signs, body weight, muscle mass, and muscle tone were reported. The results from this study were used to select the doses for a metabolism study; the doses selected represented toxicologically equivalent doses. In the metabolism study, animals (5/dose group) were treated with 4 mg/kg/day CuPT or 5 mg/kg/day ZnPT for 6 consecutive days with non-radiolabeled compound; radiolabeled test compounds were administered on Days 7 and 8. Mortality, clinical signs, body weight, food consumption, excretion profile, blood partitioning, muscle mass, muscle tone, and metabolite characterization were reported. The purpose of the metabolism study was to demonstrate that at doses that produced similar toxicity, the disposition (absorption, distribution, metabolism, and excretion) of the two test articles would be similar.

In the range-finding study, CuPT and ZnPT treatment of rats resulted in neurological signs such as irregular gait, lethargy, and paralysis. CuPT treatment resulted in a dose-dependent decrease in body weight, which was not observed with ZnPT. In the CuPT-treated animals, muscle mass and tone were greatly reduced. In the ZnPT-treated animals, equivocal effects on muscle mass and tone were noted without a clear dose-dependent effect.

Doses of 4 mg/kg/day CuPT and 5 mg/kg/day ZnPT were chosen for the metabolism study, as these doses provided the most similar toxicological effects, based on clinical signs. In the metabolism study, toxicity was shown to be similar between the 4 mg/kg/day CuPT group and the 5 mg/kg/day ZnPT group, except that the effect on muscle was slightly more severe in the CuPT group. No mortality was observed. All animals were slightly lethargic on Day 5 at 4 hours post-dosing. The only other clinical sign noted at the 4-hour observations was chromodacryorrhea in a single ZnPT rat on Day 8. At 0.5 hours post-dosing, the following findings were observed (# observed/32 observations) in both the CuPT and ZnPT groups: slightly lethargic (4-5); non-responsive (4); prostrate (1 CuPT); salivating (1-3); lachrymating (1 CuPT); or chromodacryorrhea (1 ZnPT). These signs were first observed at Days 5 or 6. It was stated that no statistical difference was detected between body weights or food consumption of the CuPT and ZnPT groups. The animals maintained their initial body weight through Day 6. After administration of the radiolabeled compound (Days 7 and 8), losses of 31 g and 8 g were noted in the CuPT and ZnPT groups, respectively. In the CuPT-treated animals, muscle mass was slightly reduced in 1 animal and greatly reduced in 3 animals; likewise, no muscle tone was noted in 3 rats and 1 rat only had moderate tone. In the ZnPT-treated animals, muscle mass was slightly reduced in 2 animals and normal in 3 rats; muscle tone was moderate in all 5 rats.

The disposition of radioactivity in the CuPT- and ZnPT-treated groups was similar, based on the excretion profile, metabolic profile, and blood compartmentalization. Total recovery was 92.5-96.5% of the administered dose (AD), with 50.5-57.9% AD isolated in the tissues and 34.9-37.6% AD found in the excreta with an additional 3.7-4.4% AD isolated in the cage rinse. Most of the dose was found in the carcass (49.5-56.9% AD) with only 1% isolated in the blood. The compounds were eliminated almost exclusively in the urine. Total recovery of the radiolabeled doses (28 hours after the first dose and 4 hours after the second dose) in excreta was 33.5-36.7% AD in urine and 0.9-1.4% AD in feces. Urinary and fecal elimination of CuPT and ZnPT as a percentage of administered doses were not statistically different between the two compounds. Generally, the HPLC profiles of urine and plasma were similar between the two compounds. Pyrithione accounted for only a very small fraction of the urinary radioactivity at all time points. The major metabolite in the plasma was identified as 2-methylsulfonyl pyridine through HPLC and GC-MS. No other metabolites were identified. No statistically significant differences were noted between the two test articles as a percentage of dose administered, nor between the blood/plasma and RBC/plasma ratios (which were approximately 1).

This study is classified as **acceptable/non-guideline**. In the Disposition study, animal received daily doses of 4 mg/kg copper omadine or 5 mg/kg zinc omadine by oral gavage for 8 days. Overall, muscle mass was reduced to a greater extent in the copper omadine –dosed animals compared with zinc omadine dosed animals. For copper omadine, at 4 mg/kg/day oral dose, four out of 5 rats show signs of reduced muscle mass and three of them were reported as greatly reduced muscle mass. At higher dose (5 mg/kg/day), only two out of five show slightly reduced muscle mass and other animals are consider normal. Likewise, greater loss of muscle tone when compared with zinc-omadine dosed animal. In conclusion, the effects may be similar, CuPT would cause more potent effects and tends to trigger the effects faster when compare with ZnPT.

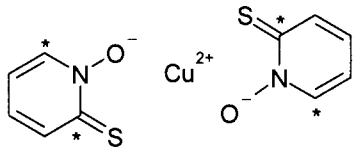
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided. It was stated that the study was conducted using best scientific practices, but was not conducted in compliance with FIFRA or OECD GLP Regulations.

I. MATERIALS AND METHODS

A. MATERIALS

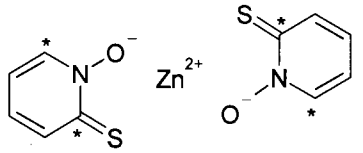
1. Test compounds

Radiolabelled test material 1: [^{14}C] Copper pyrithione
- **Radiochemical purity:** >98%
- **Specific activity:** 9.98 mCi/mmol
- **Lot/batch #:** Not reported
- **Structure:**



* indicates position of ^{14}C-label

Radiolabelled test material 2: [^{14}C] Zinc pyrithione
- **Radiochemical purity:** >98%
- **Specific activity:** 9.98 mCi/mmol
- **Lot/batch #:** Not reported
- **Structure:**



* indicates position of ^{14}C-label

Non-radiolabelled test material 1: Copper pyrithione (CuPT)
- **Description:** Olive green solid
- **Lot/batch #:** Not reported
- **Purity:** Not reported
- **CAS # of TGAI:** 14915-37-8

Non-radiolabelled test material 2: Zinc pyrithione (ZnPT)
- **Description:** White solid
- **Lot/batch #:** Not reported
- **Purity:** Not reported
- **CAS # of TGAI:** 13463-41-7

2. Vehicle: Aqueous 0.5% Darvan (sodium polynaphthalenesulfonate)

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley CD® (Crl:CD®(SD)IGS BR); females only
Age/weight on Day 1:	Age not reported; 213-243 g
Source:	Charles River Laboratories (Raleigh, NC)
Housing:	Individually in polycarbonate cages, except individually in all-glass Roth-type metabolism chambers during excreta collection
Diet:	Certified Purina Rodent Chow 5002 (Purina Mills, Inc., St. Louis, MO), *ad libitum*
Water:	Deionized water, *ad libitum*
Environmental conditions	**Temperature:** 69-76°F **Humidity:** 40-70% **Air changes:** ≥10/hr **Photoperiod:** 12 hours light/12 hours dark
Acclimation period:	At least 1 week, acclimated to metabolism cages for 1 day prior to radiolabel dose

B. STUDY DESIGN AND METHODS

1. **Group arrangements, dosing, and study purpose:** An initial range-finding study was performed to investigate the toxicity (based on mortality, clinical signs, body weights, muscle mass, and muscle tone) of CuPT and ZnPT in adult female Sprague-Dawley rats. The results from this study were used to select the doses for the following metabolism study. The doses selected represented a toxicologically equivalent dose. The animals were randomly assigned, stratified by body weight, to the test groups shown in Table 1a.

TABLE 1a. Study design for the 9-day toxicity study [a]

Test group	Dose to animal (mg/kg/day)	Actual intake (mg/kg/day)	No. female rats
		CuPT	
Control [b]	0	0	3
Low	4	4.2	3
Mid	6	6.2	3
Mid-High	9	9.1	3
High	12	12.1	3
		ZnPT	
Control [b]	0	0	3
Low	3	3.3	3
Mid	5	5.1	3
Mid-High	8	8.6	3
High	11	12.6	3

a Data were obtained from pages 19 and 33-40 of MRID 45774326.
b There was one control group with 3 rats, which served as the control for both the copper and zinc pyrithione studies.

The goal of the metabolism study was to demonstrate that at doses that produced similar toxicity, the disposition (based on preliminary assessment of absorption, metabolism, distribution, and excretion) of the two test articles was similar. Mortality, clinical signs, body weights, food consumption, muscle mass, and muscle tone were also evaluated. The animals were randomly assigned, stratified by body weight, to the test groups shown in Table 1b.

TABLE 1b. Dosing groups for the metabolism study [a]

Test article	Dose level (mg/kg)		No. female rats	Remarks
	Nominal	Actual mean [b]		
CuPT	4	3.8	5	Blood, urine, feces, and carcass were collected at termination.
ZnPT	5	5.1	5	

a Data were obtained from pages 20 and 58 of MRID 45774326.
b This group received 6 consecutive daily doses by gavage with unlabeled test article (4 mg/kg copper pyrithione or 5 mg/kg zinc pyrithione, nominally) followed by the same dose of radiolabeled test article on Days 7 and 8 by gavage. Actual mean presented in the table is an average of radiolabeled test article administered on Days 7 and 8.

Animals were dosed once daily by oral gavage (5 mL/kg) for 9 consecutive days (range-finding study) or 8 consecutive days (metabolism study). On Days 7 and 8 of the metabolism studies, radiolabeled test compound was administered. The actual dose was determined gravimetrically.

The in-life phase was March 19, 2002 through April 17, 2002.

2. **Dose preparation and analysis:** The test compounds were formulated by the Sponsor and shipped to the investigating lab. The radiolabeled compounds were supplied at concentrations of 1.0 mg/g (ZnPT) and 0.8 mg/g (CuPT). The specific activity for both radiolabeled compounds was 9.98 mCi/mmol. Radiochemical purity of the dosing solutions were >98%. Upon receipt, these formulations were stored at approximately 4°C. Stock suspensions of radiolabeled and non-radiolabeled CuPT were prepared by dissolving labeled and non-radiolabeled CuPT in acidic solution, heating, and then precipitating the CuPT with copper chloride. The identity of the copper pyrithione prepared in this way was confirmed by mass spectrometry. Labeled and non-radiolabeled ZnPT suspensions were prepared by dissolving ZnPT in acidic solution, heating, and then neutralizing to re-precipitate the ZnPT. The process purified the radiolabeled compounds and ensured identical particle size and shape for the corresponding labeled and non-radiolabeled compounds. Dose formulations were prepared by suspending appropriate concentrations of the test compounds in aqueous 0.5% Darvan. Homogeneity (top, middle, and bottom) was determined for the Day 7 and 8 radiolabeled dose solutions. Stability following 14 days of storage (temperature not specified) was determined in the low and high dose formulations of the range-finding study. Concentrations were measured in duplicate samples of all dose levels for both studies.

Results

Homogeneity (% coefficient of variation): 1-2%

Stability (% of initial): 98-106%

Concentration (% of nominal): 102-116%

The analytical data indicated that the mixing procedure was adequate. However, the variation between nominal and actual dosage to the animals was considered marginally acceptable but adequate for the purposes of these studies.

3. **Statistics:** Body weights and food consumption were compared using two-way analyses of variance for repeated measures followed by Student's-Neuman-Kuels test for multiple comparisons when appropriate. Statistical comparisons of copper and zinc pyrithione derived total radioactivity in excreta, carcass, blood, plasma, and erythrocytes (method of analysis not reported). Blood to plasma ratios and erythocyte to plasma ratios were performed by first analyzing for normality and equal variances (statistical tests not reported). When the test data sets were determined to be normally distributed and variances were equal, then the data were compared using Student's *t*-test. When data sets were determined not to be normally distributed or variances were unequal, then a nonparametric Mann-

Whitney rank sum test was performed. Statistical significance was considered to be achieved at a level of $p < 0.05$. These statistical analyses were considered appropriate.

C. METHODS

1. **Range-finding study:** Individual body weights were measured prior to treatment, daily during treatment, and at necropsy. Mortality and clinical observations were monitored twice daily. Four hours following the last dose, hind limb muscle weakness (muscle mass and tone) were evaluated in each animal by a single individual throughout the studies. The technician was unaware of each rat's dose group.

   To determine muscle mass, the technician rolled the extensor muscles of the tarsus between his thumb and forefinger and evaluated the muscle mass subjectively via digital palpation. The response was recorded as normal, slightly reduced, or greatly reduced.

   To determine muscle tone, the technician supported the subject in the air by grasping the thorax gently from behind. With the free hand, the technician gently but briskly pressed the tips of two fingers (or one finger and thumb) into the middle of the footpads of each hind limb (one digit into each footpad). As the rat extended the hind limbs, the presence/strength of the extensor response was evaluated subjectively via digital palpation. The strength of the extensor response was recorded as none, low, moderate, or high.

2. **Metabolism study**

a. **Observations and parameters examined in rats:** Individual body weights were measured prior to treatment, daily during treatment, and at necropsy. Mortality and clinical observations were monitored twice daily. Three hours following the last dose, hind limb muscle weakness (muscle mass and tone) were evaluated in each animal. The methodology was the same as in the range-finding study.

   Additionally, food consumption was measured daily for the first 6 days of dosing. Food consumption was not measured during Days 7 and 8 due to potential inconsistencies after animals were placed in metabolism cages.

b. **Collection and storage of samples:** Urine and feces were collected separately from each animal into receivers cooled over dry ice and protected from light. Urine and feces were collected prior to the first radiolabeled dose administration and at 6, 12, and 24 hours post-dose. Following the last radiolabeled dose, urine and feces were collected for 4 hours. Following euthanization, bladder urine was also collected and added to the last urine collection. At the end of the study, the cage was rinsed with water and ethanol, and the rinses were analyzed for radioactivity. The weight of urine and/or feces collected for each sample interval was measured. Aliquots of urine samples were immediately analyzed for total radioactivity, and residual urine was stored in tightly capped containers in the dark at approximately -20°C until shipped to the Sponsor for analysis of metabolic profiles.

Blood was collected from the tail vein of each animal prior to dosing and another sample was obtained at 4 hours following the last radiolabeled dose. The last blood sample was obtained by cardiac puncture prior to sacrifice by CO_2 asphyxiation and section of the diaphragm. The weight of the blood samples was measured. Packed erythrocytes and plasma were prepared from aliquots of collected whole blood samples by centrifugation. Residual aliquots of plasma were stored at approximately -20°C until shipped to the Sponsor for analysis of metabolic profiles.

The remaining carcasses were weighed and digested in 2 N ethanolic sodium hydroxide.

c. **Analysis of samples:** Samples were assayed for total radioactivity either directly or following solubilization in Soluene-350. Samples that were too dark were bleached (perchloric acid/H_2O_2,) prior to analysis by liquid scintillation counting (LSC). Control (pre-exposure) samples of urine, feces, and blood were collected during the acclimation period and analyzed for radiochemical content to determine background counts. Duplicate aliquots of urine were analyzed directly (without solubilization or bleaching) for radiochemical content. Feces were homogenized with an approximately equal mass of water. The weight of the feces homogenate was determined and weighed aliquots were solubilized in Soluene-350 prior to analysis by LSC. Whole blood, packed erythrocytes, plasma, and carcass were solubilized, bleached as necessary, and analyzed for total radioactivity by LSC.

d. **Metabolite characterization:** The urine from each dose group was pooled by animal and by time point. Analysis of pooled urine was prepared for HPLC both with and without addition of the derivatizing reagent, 2-pyridine disulfide (PDS). PDS converts the reactive pyrithione molecule to a mixed disulfide that will not absorb onto the HPLC column. Pooled urine was combined with water, either PDS (1 mg/ml in acetonitrile/water 1:1) or acetonitrile/water 1:1, and filtered through a Nylon 0.2 µm syringe filter. The samples were analyzed by HPLC with radioactivity and UV detection.

Plasma from each animal was spiked with a standard of 2-methylsulfonyl pyridine (2-MSP) and derivatized with PDS. The plasma sample was mixed with acetonitrile and 2-MSP (1.51 mg/ml in acetonitrile) on a vortex mixer and then centrifuged. The supernatant was filtered through a Nylon 0.2 µm syringe filter, diluted with water, and analyzed by HPLC.

Chromatograms were used to compare the metabolic profile of copper and zinc pyrithione. Identification was performed for only a single plasma metabolite using GC/MS.

e. **Blood to plasma ratio:** The concentration of total radioactivity in approximately equal volumes of whole blood, plasma, and packed erythrocytes (RBCs) was determined in blood samples collected at 4 hours following the last radiolabeled dose. The blood to plasma ratio was calculated by dividing the concentration of radioactivity in whole blood aliquots by the concentration of radioactivity in plasma aliquots. The whole blood hematocrit (HCT) was determined by conventional techniques for each animal from each blood sample collected prior to the first dose of test article and at 4 hours following the last radiolabeled dose. The ratio of total radioactivity in RBCs and plasma was determined by two methods: (1) the

RBC to plasma ratio was calculated from direct measurement by dividing the concentration of total radioactivity in RBC aliquots by the concentration of total radioactivity in plasma aliquots, and (2) the RBC to plasma ratio was also calculated indirectly using the following equation:

$$\frac{RBC}{C} = \frac{C_{blood} - \left[(1 - HCT) \times C_{plasma}\right]}{HCT \times C_{plasma}}$$

where C_{blood} is the concentration of total radioactivity in whole blood (dpm-eq/g), HCT is the sample hematocrit at 4 hours following the last radiolabeled dose, and C_{plasma} is the concentration of total radioactivity in plasma (dpm-eq/g). This equation allows for the calculation of total radioactivity in RBCs without possible contamination of concentrations of total radioactivity from plasma that can occur by direct measurement of RBCs.

II. RESULTS

A. RANGE-FINDING STUDY

1. Observations

a. Mortality: One 9 mg/kg/day CuPT female (#G682) was found dead before dosing Day 9.

b. Clinical signs of toxicity: All of the CuPT animals had irregular gait at 0.5 and 4 hours post-dosing by Day 7, and paralysis by Day 9. Hunched posture was frequently observed, beginning on Days 6-7 in all dose groups. Lethargy was noted at 9 mg/kg/day and above beginning on Day 6, and at 4 and 6 mg/kg/day beginning on Day 7. All symptoms worsened in a dose-dependent manner.

At 5 mg/kg/day and above, the ZnPT rats were lethargic with a slight change in gait at 0.5 and 4 hours post-dosing by Day 7, progressing to irregular gait by Day 8. The 3 mg/kg/day ZnPT animals were lethargic with a slight change in gait by Day 8, progressing to irregular gait by Day 9. Hunched posture was frequently observed. All symptoms worsened in a dose-dependent manner.

2. Body weight and body weight gain: In the CuPT-treated groups, body weight losses were observed at all doses (-29 g to -55 g treated compared to +22 g controls) in a dose dependent manner over the entire (Days 1-9) treatment period. The Sponsor stated that across all days and all dose groups, body weights were statistically lower ($p < 0.05$) compared with the vehicle controls.

TABLE 2a. Body weights and body weight gains (g) in female rats treated with CuPT by gavage for 9 days [a]					
Day (s)	Dose (mg/kg/day)				
	0	4	6	9	12
1	228±6	228±9	230±12	229±3	230±9
2	230±5	230±2	227±12	224±8	223±3
3	233±3	227±5	229±7	213±8	215±3
9	250±3	199±20	192±7	179±4	175±10
Days 1-9 [b]	22	-29	-38	-50	-55

a Data (n=3) were obtained from Table 2 on pages 41-43 in MRID 45774326. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

b Body weight gains were calculated by reviewers from mean body weights reported in the cited data. Statistical analyses were not performed.

In the ZnPT-treated groups, minor decreases in body weights were generally observed throughout the treatment period at all doses, but not in a dose-dependent manner (Table 2b). Body weight losses were observed at 5 and 8 mg/kg/day (-8 g and -3 g, respectively, compared to +22 g controls) over the entire (Days 1-9) treatment period. The Sponsor stated that across all days, body weight measurements were statistically lower ($p < 0.05$) in the 5 and 8 mg/kg/day groups compared with the vehicle controls.

TABLE 2b. Body weights and body weight gains (g) in female rats treated with ZnPT by gavage for 9 days [a]					
Day (s)	Dose (mg/kg/day)				
	0	3	5	8	11
1	228±6	226±8	228±10	229±5	226±8
2	230±5	228±5	228±9	226±4	225±6
9	250±3	234±9	220±20	226±1	234±10
Days 1-9 [b]	22	8	-8	-3	8

a Data (n=3) were obtained from Table 2 on pages 41-43 in MRID 45774326. Percent difference from controls is included in parentheses, and was calculated by the reviewers.

b Body weight gains were calculated by reviewers from mean body weights reported in the cited data. Statistical analyses were not performed.

3. **Muscle mass and tone:** In the CuPT-treated animals, muscle mass was greatly reduced in all treated animals, with the exception of a single rat at 12 mg/kg/day (slight reduction; Table 3). Likewise, muscle tone was reduced to none in all treated animals, with the exception of a single rat at 6 mg/kg/day (low) compared to high muscle tone in all controls.

In the ZnPT-treated animals, equivocal effects on muscle mass (slight reductions in 1-3/3 animals) and on muscle tone (none to high) were observed without a clear dose-dependent effect.

TABLE 3.

Functional Assessments in Rats Following Repeated Oral Administration of Copper Pyrithione (CuPT) or Zinc Pyrithione (ZPT) for 9 Days

Test Article	Dose (mg/kg/day)	Muscle Mass[a]			Muscle Tone[b]			
		A	B	C	A	B	C	D
Vehicle	0	3/3	0/3	0/3	0/3	0/3	0/3	3/3
CuPT	4	0/3	0/3	3/3	3/3	0/3	0/3	0/3
	6	0/3	0/3	3/3	2/3	1/3	0/3	0/3
	9[c]	0/2	0/2	2/2	2/2	0/2	0/2	0/2
	12	0/3	1/3	2/3	3/3	0/3	0/3	0/3
ZPT	3	2/3	1/3	0/3	1/3	0/3	1/3	1/3
	5	1/3	2/3	0/3	2/3	0/3	1/3	0/3
	8	2/3	1/3	0/3	0/3	0/3	2/3	1/3
	11	0/3	3/3	0/3	0/3	1/3	2/3	0/3

[a] Scores are number of animals affected relative to total number of animals in the group.
A = normal muscle mass.
B = slightly reduced muscle mass.
C = greatly reduced muscle mass.

[b] Scores are numbers of animals affected (extensor muscle presence/strength) relative to total number of animals in the group.
A = None
B = Low
C = Moderate
D = High

[c] One animal in this group (#G682) was found dead before dosing on Day 9.

This table was copied from page 53 (Table 4) of the study report.

B. METABOLISM STUDY

1. Observations

a. Mortality: No mortality was observed.

b. Clinical signs of toxicity: All animals were slightly lethargic on Day 5 at 4 hours post-dosing. The only other clinical sign noted at the 4-hour observations was chromodacryorrhea in a single ZnPT rat on Day 8. At 0.5 hours post-dosing, the following findings were observed (# observed/32 observations) in both the CuPT and ZnPT groups: slightly lethargic (4-5); non-responsive (4); prostrate (1 CuPT); salivating (1-3); lachrymating (1 CuPT); or chromodacryorrhea (1 ZnPT). These signs were first observed at Days 5 or 6.

2. Body weight and body weight gain:

It was stated that no statistical difference was detected between the CuPT and ZnPT groups. The animals maintained their initial body weight through Day 6 (Table 4). After administration of the radiolabeled compound (Days 7 and 8), losses of 31 g and 12 g were noted in the CuPT and ZnPT groups, respectively.

TABLE 4. Body weights and body weight gains (g) in female rats [a]	
4 mg/kg/day CuPT	
Day 1	233±5
Day 6	234±6
Day 8	203±10
Days 1-8	-30
5 mg/kg/day ZnPT	
Day 1	229±10
Day 6	233±16
Day 8	221±17
Days 1-8	-8

a Data (n=5) were obtained from Table 8 on page 60 in MRID 45774326.

3. **Food consumption:** It was stated that no statistical difference was detected between the CuPT and ZnPT groups. The mean (Days 1-6) food consumption was 73.2 g/kg and 67.7 mg/kg in the CuPT and ZnPT groups, respectively.

4. **Muscle mass and tone:** In the CuPT-treated animals, muscle mass was slightly reduced in 1 animal and greatly reduced in 3 animals (Table 5). Likewise, no muscle tone was noted in 3 rats and 1 rat only had moderate tone. One animal had normal muscle mass, and one animal had normal muscle tone.

   In the ZnPT-treated animals, muscle mass was slightly reduced in 2 animals and normal in 3 rats. Muscle tone was moderate in all 5 rats.

TABLE 5.

Functional Assessments in Rats Following Repeated Oral Administration of Copper Pyrithione (CuPT) or Zinc Pyrithione (ZPT) for 8 Days

Test Article	Dose (mg/kg/day)	Muscle Mass[a]			Muscle Tone[b]			
		A	B	C	A	B	C	D
CuPT	4	1/5	1/5	3/5	3/5	0/5	1/5	1/5
ZPT	5	3/5	2/5	0/5	0/5	0/5	5/5	0/5

[a] Scores are number of animals affected relative to total number of animals in the group.
A = normal muscle mass.
B = slightly reduced muscle mass
C = greatly reduced muscle mass.

[b] Scores are numbers of animals affected (extensor muscle presence/strength) relative to total number of animals in the group.
A = None
B = Low
C = Moderate
D = High

This table was copied from page 65 (Table 11) of the study report.

5. **Mass balance:** For the CuPT group, total recovery of radioactivity was 96.5% of the administered dose (AD), with 57.9% AD recovered in the tissues, 34.9% AD excreted, and 3.7% AD recovered in the cage rinse (Table 6). For the fraction excreted, 33.5% AD was found in the urine, and 1.4% AD was recovered in the feces. Similarly in the ZnPT group, total recovery of radioactivity was 92.5% AD, with 50.5% AD recovered in the tissues, 37.6% AD excreted, and 4.4% recovered in the cage rinse. For the fraction excreted, 36.7% AD was found in the urine, and 0.9% AD was recovered in the feces. In the tissues, the majority of the radioactivity was found in the carcass (56.9% AD CuPT; 49.5% AD ZnPT), with a small fraction (approximately 1% AD in both groups) found in the blood.

TABLE 6.

Excretion and Distribution of Total Radioactivity Following Repeated Oral Administration of Copper Pyrithione (CuPT) or Zinc Pyrithione (ZPT) for 8 Days

End of Collection Period (h)	Cumulative Percent Dose Excreted[a]					
	Urine		Feces		Total	
	CuPT	ZPT	CuPT	ZPT	CuPT	ZPT
8	2.4 ± 2.2	5.9 ± 4.6	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 2.2	5.9 ± 4.6
12	16.6 ± 6.7	17.0 ± 4.8	0.2 ± 0.3	0.0 ± 0.0	16.7 ± 6.9	17.0 ± 4.8
24	30.4 ± 6.3	31.1 ± 5.1	1.2 ± 1.5	0.6 ± 0.4	31.5 ± 7.0	31.7 ± 5.4
28[b]	33.5 ± 6.9	36.7 ± 6.3	1.4 ± 1.7	0.9 ± 0.5	34.9 ± 7.2	37.6 ± 6.5

Distribution in Tissues[a]

Tissue	DPM-eq per g Tissue		% Dose in Total Tissue	
	CuPT	ZPT	CuPT	ZPT
Blood[c]	75764 ± 19372	98910 ± 14354	1.1 ± 0.2	1.0 ± 0.2
Carcass	101129 ± 9967	109924 ± 11931	56.9 ± 5.5	49.5 ± 5.6

Overall Percent Dose Recovered[a]

Test Article	% Dose Recovered in Tissues	% Dose Recovered in Cage Rinse	% Dose Excreted[d]	Overall % Dose Recovered
CuPT	57.9 ± 5.3	3.7 ± 2.4	34.9 ± 7.2	96.5 ± 2.1
ZPT	50.5 ± 5.5	4.4 ± 2.2	37.6 ± 6.5	92.5 ± 3.9

[a] All values expressed as mean ± S.D. (N=5). The target doses were 4 mg/kg CuPT and 5 mg/kg ZPT. The actual mean doses delivered were 3.9 ± 0.1 mg CuPT/kg and 5.1 ± 0.1 mg ZPT/kg. Cumulative percent of doses are relative to the summation of radiolabeled doses that animals received on Day 7 and Day 8.

[b] Includes results from 28-h urine collection and residual urine in bladder.

[c] Percent of dose in blood was calculated assuming blood volume is 7.4% of body weight (Brown et al., 1997).

[d] Includes urine and feces.

This table was copied from page 67 (Table 13) of the study report.

6. **Metabolite characterization:** Generally, the HPLC profiles of urine and plasma were similar between the two compounds. Pyrithione accounted for only a very small fraction of the urinary radioactivity at all time points. The major plasma metabolite for both compounds was identified as 2-methylsulfonyl pyridine (2-MSP) using HPLC and GC-MS analysis. No other metabolites were identified.

7. **Blood to plasma ratio:** No statistically significant differences were noted between the two test articles as a percentage of dose administered (Table 7), nor between the blood/plasma and RBC/plasma ratios (which were approximately 1).

TABLE 7.

Distribution of Total Radioactivity in Blood Fractions Following Repeated Oral Administration of Copper Pyrithione (CuPT) or Zinc Pyrithione (ZPT) for 8 Days

Test Article	Animal Ear Tag #	dpm-eq/g tissue			B/P	RBC/P[a]	RBC/P[b]
		Blood	Plasma	RBC			
CuPT	G698	80491	80174	80709	1.0	1.0	1.0
	G706	70411	73244	67435	1.0	0.9	0.9
	G709	78745	86743	72083	0.9	0.8	0.8
	G700	101406	93621	108609	1.1	1.2	1.2
	G704	47768	44429	50564	1.1	1.1	1.2
	Mean	75764	75642	75880	1.0	1.0	1.0
	SD	19372	19020	21338	0.1	0.1	0.2
	%CV	26	25	28	7	14	16
ZPT	G705	99316	95991	99637	1.0	1.0	1.1
	G708	99047	96224	101627	1.0	1.1	1.1
	G702	79635	77675	80728	1.0	1.0	1.1
	G699	120044	125369	117127	1.0	0.9	0.9
	G701	96511	98835	92619	1.0	0.9	0.9
	Mean	98910	98819	98347	1.0	1.0	1.0
	SD	14354	17079	13307	0.0	0.1	0.1
	%CV	15	17	14	4	6	9

[a] Estimated from direct measurement of radioactivity in packed RBCs and plasma.

[b] Estimated indirectly from whole blood and plasma concentrations of total radioactivity using Eq. (1). See Section 4.9.

This table was copied from page 68 (Table 14) of the study report.

III. DISCUSSION AND CONCLUSIONS

A. REVIEWER COMMENTS: CuPT and ZnPT treatment of rats resulted in neurological signs such as irregular gait, lethargy, and paralysis. The CuPT treatment resulted in dose-dependent body weight losses, which was not observed with ZnPT treatment. In the CuPT-treated animals, muscle mass and tone were greatly reduced. In the ZnPT-treated animals, equivocal effects on muscle mass and tone were noted without a clear dose-dependent effect.

In the metabolism study, doses of 4 mg/kg/day CuPT and 5 mg/kg/day ZnPT were chosen, as these provided the most similar toxicological effects. This similarity was based on clinical signs. All the 4 mg/kg/day CuPT animals had irregular gait by Day 7 and paralysis by Day 9 at 0.5 and 4 hours post-dose. All the 5 mg/kg/day ZnPT animals had a slight change in gait by Day 7 and were lethargic with an irregular gait by Day 9 at 0.5 and 4 hours post-dose.

No mortality was observed. All animals were slightly lethargic on Day 5 at 4 hours post-dosing. The only other clinical sign noted at the 4-hour observations was chromodacryorrhea

in a single ZnPT rat on Day 8. At 0.5 hours post-dosing, the following findings were observed (# observed/32 observations) in both the CuPT and ZnPT groups: slightly lethargic (4-5); non-responsive (4); prostrate (1 CuPT); salivating (1-3); lachrymating (1 CuPT); or chromodacryorrhea (1 ZnPT). These signs were first observed at Days 5 or 6. No statistical difference was detected between the CuPT and ZnPT dose groups based on body weights. The animals maintained their initial body weight through Day 6. After administration of the radiolabeled compound (Days 7 and 8), losses of 31 g and 8 g were noted in the CuPT and ZnPT groups, respectively. It was stated that no statistical difference was detected in the food consumption of the groups. In the CuPT-treated animals, muscle mass was slightly reduced in 1 animal and greatly reduced in 3 animals; likewise, no muscle tone was noted in 3 rats and 1 rat only had moderate tone. In the ZnPT-treated animals, muscle mass was slightly reduced in 2 animals and normal in 3 rats; muscle tone was moderate in all 5 rats. In the Disposition study, animal received daily doses of 4 mg/kg copper omadine or 5 mg/kg zinc omadine by oral gavage for 8 days. Overall, muscle mass was reduced to a greater extent in the copper omadine – dosed animals compared with zinc omadine dosed animals. For copper omadine, at 4 mg/kg/day oral dose, four out of 5 rats show signs of reduced muscle mass and three of them were reported as greatly reduced muscle mass. At higher dose (5 mg/kg/day), only two out of five show slightly reduced muscle mass and other animals are consider normal. Likewise, greater loss of muscle tone when compared with zinc-omadine dosed animal. In conclusion, the effects may be similar, CuPT would cause more potent effects and tends to trigger the effects faster when compare with ZnPT.

The disposition of the CuPT-treated animals compared to the ZnPT-treated animals was similar based on the excretion profile, metabolic profile, and blood compartmentalization. For the CuPT group, total recovery of radioactivity was 96.5% of the AD, with 57.9% AD recovered in the tissues, 34.9% AD excreted, and 3.7% AD recovered in the cage rinse. For the fraction excreted, 33.5% AD was found in the urine, and 1.4% AD was recovered in the feces. Similarly in the ZnPT group, total recovery of radioactivity was 92.5% AD, with 50.5% AD recovered in the tissues, 37.6% AD excreted, and 4.4% recovered in the cage rinse. For the fraction excreted, 36.7% AD was found in the urine, and 0.9% AD was recovered in the feces. In the tissues, the majority of the radioactivity was found in the carcass (56.9% AD CuPT; 49.5% AD ZnPT), with a small fraction (approximately 1% AD in both groups) found in the blood. Generally, the HPLC profiles of urine and plasma were similar between the two compounds. Pyrithione accounted for only a very small fraction of the urinary radioactivity at all time points. The major plasma metabolite for both compounds was identified as 2-MSP. No other metabolites were identified. No statistically significant differences were noted between the two test articles as a percentage of dose administered, nor between the blood/plasma and RBC/plasma ratios (~1).

A. **CONCLUSIONS:** The deposition study indicate the radioactivity in the CuPT- and ZnPT-treated groups was similar (based on preliminary assessment of absorption, metabolism, distribution, and excretion). In the 9-day oral toxicity study, overall, CuPT would cause more potent effects (muscle mass reduction) and tends to trigger the effects faster when compare with ZnPT.

Sign-off Date: 02/15/11 DP Barcode Nos.: D375749 and D369393

TXR No.: 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D. **Signature:** Jonathan Chen
RASSB, Antimicrobial Division **Date:** 01/06/2011
EPA Secondary Reviewer: Jenny Tao **Signature:**
RASSB, Antimicrobial Division **Date:** 1-6-11
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study in Rats (gavage); OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 088001 **DP BARCODE:** 380528

TEST MATERIAL (PURITY): Copper pyrithione (99.6%)

SYNONYMS: Copper-2-pyrithio-1-oxide; Copper Omadine

CITATION: Wood, E. (2000) Copper pyrithione: oral gavage teratology study in the rat. Safepharm Laboratories Limited, Derby, UK. Laboratory Project No.: 696/035, February 17, 2000. MRID 48158006. Unpublished.

Wood, E. (1999) Copper pyrithione: preliminary oral gavage teratology study in the rat. Safepharm Laboratories Limited, Derby, UK. Laboratory Project No.: 696/034, November 16, 1999. MRID 48158005. Unpublished.

SPONSOR: Yoshitomi Fine Chemicals Ltd., Osaka, Japan

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 48158006), copper pyrithione (99.6%; batch/lot #990128) in 1% carboxymethyl cellulose was administered via daily oral gavage in a dose volume of 10 mL/kg to 25 time-mated Sprague-Dawley rats/dose group at doses of 0, 0.5, 1.5, or 3 mg/kg/day from gestation days (GD) 6-15. On GD 20, all dams were euthanized; each dam's uterus was removed via cesarean section and its contents examined. Fetuses were examined for external, visceral, and skeletal malformations and variations.

In the 3 mg/kg/day group, body weight gains (relative to GD 6, start of dosing) were decreased by 6-31% throughout the dosing period and food consumption was decreased ($p<0.01$) by 10% during GD 6-9.

The maternal LOAEL is 3 mg/kg/day, based on decreases in body weight gain and food consumption during the dosing period. The maternal NOAEL is 1.5 mg/kg/day.

There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses and no effects of treatment on the numbers of litters, live fetuses, or total resorptions. Similarly, fetal weights, sex ratio, and pre- and post-implantation losses were unaffected by treatment.

There were no treatment-related external, visceral, or skeletal variations or malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 3 mg/kg/day.

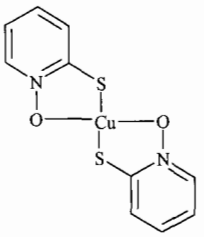
Although the developmental LOAEL was not observed, the dose rationale was considered appropriate based on the results of the range-finding study submitted concurrently (MRID 48158005). Therefore, the current study is classified **acceptable/guideline** and satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Copper pyrithione

Description:	Green powder
Batch/Lot #:	990128
Purity:	99.6% (based on the Certificate of Analysis for Lot 990128, Copper Pyrithione)
Compound stability:	The test substance was stable in the vehicle for up to 2 hours.
CAS #:	14915-37-8
Structure:	

2. Vehicle: 1% Carboxymethyl cellulose

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley CD
Age/group mean weight at GD 6:	Age not reported; 266-274 g
Source:	Charles River (UK) Ltd. (Margate, Kent, UK)
Housing:	Individually in polypropylene cages with solid floors and stainless steel grid tops
Diet:	Rat and Mouse VRFI-C Diet (Charles River UK Ltd., Margate, Kent, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 19-23°C **Humidity:** 40-70% **Air changes:** ≥15/hr **Photoperiod:** 12 hours light/12 hours dark
Acclimation period:	Approximately 3-5 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: June 11, 1999 End: July 7, 1999

2. Mating: Sexually mature, nulliparous females were mated with males of the same strain at the supplier's facility. The day on which positive evidence of mating was observed was designated gestation day (GD) 0. The rats were delivered to the performing laboratory between GD 1-3.

3. Animal assignment: The mated females were randomly assigned (stratified by body weight) to the test groups presented in Table 1 below.

TABLE 1. Animal Assignment[a]				
Dose (mg/kg/day)	0	0.5	1.5	3.0
No. females	25	25	25	25

[a] Data were obtained from pages 19 and 20 of the study report.

4. **Dose selection rationale:** The doses for the current study were selected based on the results of a preliminary range-finding study (MRID 48158005) submitted concurrently. The results of the range-finding study are summarized in the Appendix of this DER.

5. **Dose preparation, administration, and analysis:** For each concentration, the appropriate amount of the test material was weighed into a glass jar and mixed with a small amount of the vehicle (1% carboxymethyl cellulose) to form a paste. Additional vehicle was added to achieve the appropriate concentration. The dose formulations were prepared daily and used immediately following preparation. The dose suspensions were administered to the animals daily from GD 6-15 (inclusive) via oral gavage in a dose volume of 10 mL/kg body weight. Dose volumes were adjusted based upon the most recent individual body weights. Homogeneity (top, middle, and bottom) and stability were confirmed at concentrations of 0.05, 0.2, and 0.5 mg/mL (which bracket those concentrations used in the current study). Concentration analyses were performed on samples of each dose level collected at the beginning, middle, and end of the study.

Results

Homogeneity (%CV): 0.47-1.11%

Stability (% of initial following 2 hour storage): 98-101%

Concentration (% of nominal):

Concentration (mg/mL)	% of nominal
0.05	89-121%
0.15	83-104%
0.3	90-110%

The results of the concentration analyses of the dose formulations from Day 1 of dosing were in excess of the nominal concentrations. Therefore, additional analyses were performed on Day 2. The results of the subsequent analyses indicated that the mixing procedure was adequate and the variation between the nominal and actual dosage to the animals was marginally acceptable.

C. OBSERVATIONS

1. **Maternal observations and evaluations:** Cage-side examinations were conducted twice daily during weekdays and once daily on holidays and weekends to check for mortality and moribundity. All females were checked for clinical signs of toxicity one hour post-dosing

throughout the dosing period, and once daily from the day of delivery throughout gestation. Individual body weights were recorded on GD 3, 6-9, 12, 15, 18, and 20. Individual food consumption was recorded for each rat for GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20, and mean daily food consumption (g/rat/day) was calculated from these data for these intervals. On GD 20, all of the surviving dams were euthanized by carbon dioxide asphyxiation followed by cervical dislocation and subjected to a gross necropsy. The ovaries and uteri of pregnant females were removed and the gravid uterus weight, number of corpora lutea, and number, position, and type of implantations were recorded. Implantation types were divided into early resorptions, late resorptions, live fetuses, and dead fetuses.

2. **Fetal evaluations:** The fetuses were euthanized by subcutaneous injection of sodium pentobarbitone. Alternate fetuses were identified using a marker and placed in Bouin's fixative. Approximately half of the fetuses from each litter were selected for visceral alterations and processed according to the methods of Barrow and Taylor.[1] These fetuses were placed in 90% industrial methylated spirits (IMS) in distilled water and examined for visceral anomalies under a low power binocular microscope. The remaining fetuses were identified using color codes wires and placed in 70% IMS in distilled water. The fetuses were eviscerated, processed, and the skeletons stained with Alizarin Red S, according to the methods of Dawson.[2] After staining, the specimens were examined for skeletal development and anomalies. External, visceral, and skeletal findings were classified as variations or malformations.

D. DATA ANALYSIS

1. **Statistical analyses:** The following statistical procedures were performed.

Parameter	Statistical test
Maternal body weight gains Maternal food consumption	Levene's test for homogeneity of variances followed by a one-way analysis of variance (ANOVA). If ANOVA was significant ($p<0.05$), pair-wise comparisons of the treated groups with the controls were conducted using Dunnett's test. If the variances were unequal, Dunnett's T_3 comparison test was performed.
Caesarian necropsy parameters Fetal parameters	Kruskal-Wallis non-parametric ANOVA. If ANOVA was significant, a subsequent pairwise analysis of control values versus treated values was performed using the Mann-Whitney 'U' test.
Skeletal findings Visceral findings	Kruskal-Wallis non-parametric ANOVA followed by the Terpstra-Jonckheere test for trends in ordered alternatives.

Significance was denoted at $p<0.05$ and 0.01. It was not stated whether the assumption of normal distribution of the data was tested prior to proceeding with parametric analyses. Otherwise, the statistical analyses were considered appropriate.

1 Barrow, V. M. and W.J. Taylor (1969). A rapid method for detecting malformations in rat fetuses. *Journal of Morphology* 127, 291-306.

2 Dawson, A.B. (1926). A note on staining of the skeleton of cleared skeletal specimens with Alizarin Red S. *Stain Technnol.* 1, 123-4.

2. **Indices:** The following indices were reported:

   Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea × 100

   Post-implantation loss (%) = (# implantations - # live fetuses)/ # implantations × 100

3. **Historical control data:** Historical control data for external, visceral, and skeletal findings were provided in Appendix XIV on pages 191-195 of the study report.

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and clinical signs of toxicity:** In the 3 mg/kg/day group, one dam (# 92) was sacrificed *in extremis* on GD 12. Prior to death, this animal exhibited respiratory distress. At necropsy, this dam displayed red/brown fluid in the thoracic cavity and fibrous adhesions involving the lungs, heart, and thorax. The cause of death was attributed to dosing trauma. All other animals survived until scheduled termination, and no treatment-related clinical signs of toxicity were observed.

2. **Body weight:** Selected maternal body weight and body weight gain data are presented in Table 2. There were no significant differences in body weights noted at any dose compared to controls. However, at 3 mg/kg/day, cumulative body weight gains (relative to GD 6) were decreased by 29-31% on GD 8 and 9, and remained decreased by 7-9% compared to controls throughout the study. Additionally, body weight gain and corrected (for gravid uterine weight) body weight gain (both calculated by reviewers) for the overall (GD 3-20) study were decreased by 7 and 17%, respectively, compared to controls. No treatment-related differences in body weight gains were observed in the 0.5 or 1.5 mg/kg/day dams.

TABLE 2. Selected mean (±SD) maternal body weights and body weight gains (g)[a]

Gestation day (GD)	Dose in mg/kg/day [# dams]			
	0 [23]	0.5 [24]	1.5 [19]	3 [24]
Body weights				
GD 3	238 ± 19.9	245 ± 20.0	250 ± 17.0	243 ± 18.1
GD 6	266 ± 17.9	273 ± 16.9	274 ± 16.1	269 ± 16.8
GD 15	320 ± 25.5	329 ± 19.5	332 ± 21.6	319 ± 20.2
GD 20	390 ± 32.4	400 ± 24.2	407 ± 26.4	384 ± 30.4
Gravid uterine weight	71.06 ± 16.08	75.57 ± 14.22	80.81 ± 9.30	73.54 ± 14.04
GD 20 (corrected)[b]	318.9	324.4	326.2	310.5
Body weight gains				
GD 6-7	4.7 ± 3.6	4.7 ± 3.8	5.4 ± 4.6	4.4 ± 4.2 (↓6)
GD 6-8	8.5 ± 5.5	8.4 ± 5.7	8.3 ± 6.5	5.9 ± 4.9 (↓31)
GD 6-9	14.0 ± 5.3	13.1 ± 5.3	13.3 ± 6.5	9.9 ± 5.9 (↓29)
GD 6-12	35.4 ± 9.1	35.3 ± 8.1	36.1 ± 9.2	33.0 ± 7.6 (↓7)
GD 6-15 (treatment)	54.3 ± 14.6	56.6 ± 8.7	57.8 ± 11.9	49.8 ± 7.6 (↓8)
GD 6-18	92.9 ± 18.3	94.8 ± 12.2	97.6 ± 17.1	84.5 ± 12.6 (↓9)
GD 6-20	124.6 ± 20.2	127.2 ± 15.5	133.1 ± 16.6	115.2 ± 19.6 (↓8)
Overall gain (GD 3-20)[c]	152	155	157	141 (↓7)
Overall gain (corrected)[b]	80.9	79.4	76.2	67.5 (↓17)

[a] Data were obtained from Tables 1, 2, and 5 on pages 38, 39, and 42 of the study report. Percent differences from the control group, calculated by the reviewers, are included in parentheses.

[b] Corrected for gravid uterine weight. Calculated by reviewers using data within this table.

[c] Calculated by reviewers using data within this table.

3. **Food consumption:** Maternal food consumption data are presented in Table 3. Food consumption for GD 6-9 was decreased ($p<0.01$) by 10% at 3 mg/kg/day compared to controls. There were no other significant differences in food consumption.

TABLE 3. Selected mean (±SD) maternal food consumption (g/animal/day)[a]

Gestation day (GD)		Dose in mg/kg/day [# dams]			
		0 [23]	0.5 [24]	1.5 [19]	3 [24]
Pre-treatment	GD 3-6	27.3 ± 1.8	28.3 ± 1.9	27.9 ± 2.8	27.7 ± 2.4
Treatment	GD 6-9	26.2 ± 2.6	26.4 ± 2.6	25.4 ± 3.0	23.5 ± 3.4** (↓10)
	GD 9-12	28.0 ± 2.9	28.2 ± 2.3	28.0 ± 2.9	26.9 ± 3.0
	GD 12-15	29.6 ± 4.5	30.5 ± 2.2	30.2 ± 3.0	29.0 ± 3.4
	GD 15-18	31.7 ± 3.9	31.8 ± 2.9	32.2 ± 3.5	30.7 ± 2.5
	GD 18-20	32.1 ± 2.9	31.4 ± 2.3	32.8 ± 3.2	31.7 ± 3.3

[a] Data were obtained from Table 3 on page 40 of the study report. Percent differences from the control group, calculated by the reviewers, are included in parentheses.

** Significantly different from the control group at $p<0.01$

4. **Gross pathology:** No treatment-related gross findings were observed at any dose.

5. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 4. At 1.5 mg/kg/day, the number of litters was decreased (19 treated vs. 23 controls); however, this finding was unrelated to dose. There were no abortions, premature deliveries, complete

litter resorptions, or dead fetuses and no effects of treatment on the numbers of litters, live fetuses, or total resorptions. Similarly, fetal weights, sex ratio, and pre- and post-implantation losses were unaffected by treatment.

TABLE 4. Cesarean section observations[a]

Observation	Dose (mg/kg/day)			
	0	0.5	1.5	3
# Animals assigned (mated)	25	25	25	25
# Animals pregnant	23	24	19	24
Pregnancy rate (%)[b]	92	96	76	96
# Non-pregnant[b]	2	1	6	1
Maternal wastage				
No. died	0	0	0	1
No. died pregnant	0	0	0	0
No. died nonpregnant	0	0	0	1
No. aborted	0	0	0	0
No. premature delivery	0	0	0	0
Total no. corpora lutea[c]	352	393	299	384
Corpora lutea/dam	15.3 ± 2.3	16.4 ± 2.2	15.7 ± 2.1	16.0 ± 2.0
Total no. implantations[c]	299	344	276	329
Implantations/dam	13.0 ± 2.4	14.3 ± 2.2	14.5 ± 1.8	13.7 ± 2.7
Total no. litters	23	24	19	24
Total no. live fetuses[c]	280	319	270	317
Live fetuses/dam	12.2 ± 2.8	13.3 ± 2.9	14.2 ± 1.9	13.2 ± 2.7
Total no. dead fetuses[d]	0	0	0	0
Dead fetuses/dam[b]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total no. resorptions[c]	19	25	6	12
Total no. resorptions/dam	0.83 ± 1.37	1.04 ± 2.85	0.32 ± 0.58	0.50 ± 0.93
Complete litter resorptions	0	0	0	0
Mean fetal weight (g)	3.75 ± 0.42	3.80 ± 0.27	3.84 ± 0.24	3.65 ± 0.25
Sex ratio (% male)	50.6 ± 12.4	42.5 ± 15.4	48.4 ± 14.1	48.0 ± 10.3
Pre-implantation loss (%)	15.1 ± 11.8	12.2 ± 10.4	7.3 ± 7.4	13.5 ± 16.9
Post-implantation loss (%)	7.2 ± 11.6	6.6 ± 16.0	2.3 ± 4.1	3.5 ± 6.6

[a] Data were obtained from Tables 4 and 5 on pages 41 and 42 of the study report.

[b] Calculated by the reviewers from data presented in this table.

[c] Tabulated by the reviewers from individual data presented in Appendix V on pages 81-88 of the study report.

[d] The reviewers determined that there were no dead fetuses in each group because the total number of live fetuses in each group + the total number of resorptions were equivalent to the total number of implantations (i.e., all post-implantation loss was accounted for by resorptions and not fetal death).

B. DEVELOPMENTAL TOXICITY

1. **External examinations:** No treatment-related external malformations or variations were observed. One fetus (from Dam # 97 in the 3 mg/kg/day group) was found to have agnathia and microstomia. However, this finding was an isolated event and was considered unrelated to treatment.

2. **Visceral examinations:** Visceral findings are presented in Table 5. There were no

significant treatment-related differences in the proportions of fetuses with visceral findings. There were no treatment-related effects on the type or incidence of visceral findings. No visceral malformations were observed. The visceral variations noted were within the historical control ranges provided, unrelated to dose, and/or of no toxicological significance.

TABLE 5. Visceral findings (mean % fetuses affected/litter)[a]

Observation	Dose (mg/kg/day)				Historical Control Ranges[b]
	0	0.5	1.5	3	
No. fetuses examined	147	165	141	164	
No. fetuses (litters) affected	47 (18)	50 (19)	20 (14)	46 (15)	
Variations					
Eyes - Ovoid lens(es)	3.9	3.7	2.4	5.4	1.4 to 5.1
Brain - Enlarged/dilated ventricle(s)	3.2	3.8	1.3	1.6	0.0 to 4.6
Thymus – Undescended lobe(s)	5.2	3.2	0.7	2.8	4.5 to 8.5
Kidney – Unilateral/bilateral small/no development of renal papillae	18.7	23.0	8.4	18.8	12.3 to 20.1
Unilateral/bilateral increased renal pelvic cavitation	4.7	3.4	1.2	3.8	1.1 to 3.3
Unilateral/bilateral kinked and/or dilated ureter(s)	17.2	11.9	4.8*	11.7	3.5 to 16.0
General – Marked subcutaneous edema	---	---	0.8	---	0.0 to 0.9

[a] Data were obtained from Table 7 on pages 44 and 45 of the study report.

[b] Data obtained from Appendix XIV on pages 191 and 192 of the study report.

--- No animals affected (i.e., zero incidence)

* Significantly different from the control group at $p<0.05$

3. **Skeletal examination:** Skeletal findings are presented in Table 6. There were no treatment-related skeletal findings. There were no skeletal malformations. The skeletal findings noted were within the historical control ranges provided, unrelated to dose, and/or of no toxicological significance.

TABLE 6. Skeletal findings (mean % fetuses affected/litter)[a]

Observation	Dose (mg/kg/day)				Historical Control Ranges[b]
	0	0.5	1.5	3	
No. fetuses examined	133	154	129	153	
No. fetuses (litters) affected	99 (23)	119 (24)	89 (19)	131 (24)	
Variations					
Cranium – Irregular ossification, one bone	19.3	21.3	12.8	21.9	0.5 to 10.4
Incomplete ossification, one bone	23.8	20.8	13.6	23.7	8.2 to 29.0
Irregular ossification, multiple bones	8.3	11.2	4.7	9.1	0.6 to 6.3
Incomplete ossification, multiple bones	13.0	6.2	3.0	10.4	6.2 to 13.4
Hyoid – No ossification	16.8	12.1	5.8	13.8	7.0 to 19.3
Vertebrae – One cervical centrum ossified	23.4	25.8	25.1	18.4	NR
Multiple cervical centrum ossified	8.2	3.7	12.1	2.7	NR
One thoracic centrum semi-bipartite	17.1	17.9	11.0	26.0	5.4 to 27.3
Multiple thoracic centrum semi-bipartite	19.7	12.2	9.9	14.7	1.2 to 8.5

[a] Data were obtained from Table 9 on pages 47-52 of the study report.

[b] Data obtained from Appendix XIV on page 194 of the study report.

NR Not reported

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** It was concluded that the maternal LOAEL was 3 mg/kg/day based on decreased food consumption and relative body weight gains during the first 3 days of gestation. The test material had no effect on any developmental parameters at any dose level. Therefore, the developmental LOAEL was not observed and the developmental NOAEL was 3 mg/kg/day.

B. **REVIEWER COMMENTS**

1. **Maternal toxicity:** In the 3 mg/kg/day group, body weight gains (relative to GD 6, start of dosing) were decreased by 6-31% throughout the dosing period and food consumption was decreased ($p<0.01$) by 10% during GD 6-9.

   No treatment-related findings were observed in the 0.5 or 1.5 mg/kg/day dams.

   The maternal LOAEL is 3 mg/kg/day, based on decreased body weight gains and food consumption during dosing. The maternal NOAEL is 1.5 mg/kg/day.

2. **Developmental toxicity**

a. **Deaths/resorptions:** There were no abortions, premature deliveries, complete litter resorptions, or dead fetuses and no effects of treatment on the numbers of litters, live fetuses, or total resorptions.

b. **Altered growth:** There were no effects of treatment on growth or development of the fetuses. Fetal weights of the treated groups were comparable to controls, and there were no treatment-related effects on ossification of the skeleton.

c. **Developmental variations:** There were no treatment-related external, visceral, or skeletal variations.

d. **Malformations:** There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL was not observed. The developmental NOAEL is 3 mg/kg/day.

Although the developmental LOAEL was not observed, the dose rationale was considered appropriate based on the results of the range-finding study submitted concurrently (MRID 48158005). Therefore, this study is classified **acceptable/guideline** and satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

C. **STUDY DEFICIENCIES:** No deficiency was noted

APPENDIX

Preliminary Oral Gavage Teratology Study in the Rat

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive teratology study in rats (MRID 48158006).

In a preliminary teratology study (MRID 48158005), copper pyrithione (purity not reported; Batch/Lot # not reported) in 1% carboxymethyl cellulose was administered via daily oral gavage in a dose volume of 10 mL/kg to 8 female Sprague-Dawley rats/dose at doses of 0, 2, 10/5, or 50 mg/kg/day from either gestation day (GD) 5-14 or 7-16.

At 50 mg/kg/day, all of the females were sacrificed *in extremis* following a single dose due to the severity of clinical signs (contraction of the abdominal muscles with extension of the lower abdomen, dark feces, hunched posture, and decreased respiratory rate) observed.

At 10/5 mg/kg/day, 4 of 8 females were sacrificed *in extremis* following two doses, and the dose level was reduced to 5 mg/kg/day for the remainder of the dosing period. Additionally, another female was sacrificed *in extremis* on GD 11 due to blood being observed in the mouth following dosing. This death was likely due to dosing trauma. Clinical signs observed at this dose included contraction of the abdominal muscles, avoidance of use of hindlimbs, piloerection, and hunched posture. Body weights were decreased by 4-12% throughout the dosing period. Food consumption was decreased by 13-34% during the dosing period.

At 2 mg/kg/day, one female was found dead on GD 9. There were no prior clinical signs noted, and this death was likely due to dosing trauma. No significant treatment-related findings were noted at this dose.

No significant differences in pre- or post-implantation loss or any uterine parameters were observed at any dose.

The maternal LOAEL is 5 mg/kg/day, based on clinical signs of toxicity, decreased body weights, and decreased food consumption. The maternal NOAEL is 2 mg/kg/day.

No treatment-related differences in external fetal anomalies, fetal weight, placental weight, or gravid uterine weight were observed at necropsy.

The developmental LOAEL was not observed. The developmental NOAEL is 5 mg/kg/day.

This study is classified as **acceptable/non-guideline.**

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance, statements were provided.

Sign-off Date: 02/15/11 DP Barcode Nos.: D375749 and D369393 TXR No.: 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D. **Signature:** Jonathan Chen
Registration Branch, Antimicrobial Division **Date:** 02/14/2011
EPA Secondary Reviewer: Steve Malish, Ph.D. **Signature:** Steven J. Malish
Registration Branch, Antimicrobial Division **Date:** 2/15/11

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity – Oral (gavage) Study in Rats, OPPTS 870.6200; (OECD 424).

PC CODE: 088001 **DP BARCODE:** D339760

TEST MATERIAL (PURITY): Copper Omadine (96.8% a.i.)

SYNONYMS: Copper pyrithione; Copper 2-pyridine, CuPT

CITATION: Barnett, J. (2006). Oral (gavage) Subchronic Neurotoxicity Study of Copper Omadine (CuPT) in Rats. Charles River Laboratories, Preclinical Services, Horsham, PA. Laboratory Project ID: AEN00008, December 29, 2006. MRID 47023701. Unpublished.

SPONSOR: Arch Chemicals, Inc., 350 Knotter Drive, Cheshire, CT 06410-0586

EXECUTIVE SUMMARY - In a subchronic neurotoxicity study (MRID 47023701), copper omadine (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was administered orally via gavage (10 mL/kg) once daily to 16 Sprague-Dawley rats/sex/dose at doses of 0 and 2.25 mg/kg/day and to 10 rats/sex/dose at 0.5 and 1.25 mg/kg/day for 91 consecutive days. At the end of the dosing period, a subset of 10 rats/sex/dose from the control and 2.25 mg/kg/day groups were allowed to recover for an additional 6 weeks. Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all animals at pre-dosing and Weeks 2, 4, 8, and 13. At study termination, 5 rats/sex/group were anesthetized and perfused *in situ* for neuropathological examination. The tissues from the perfused animals in the control and 2.25 mg/kg/day groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Acceptable positive control data were provided.

No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. One female (#16197) was sacrificed *in extremis* on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material in the range finding study. An increased ($p \leq 0.01$) incidence of slightly reduced

hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females. During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of Compound Motor Action Potential (CMAP) (decr 28%) that remained reduced, but to a lesser extent (decr 13%), following the 6-week recovery period. Treatment-related neuropathological were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis.

At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in 4/10 males and 3/10 females. This not-toxicological effects may have been caused by irritation of the oral mucosa.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

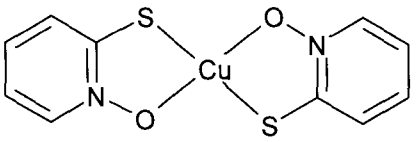
The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200) for a subchronic neurotoxicity study in rats.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Copper omadine

Description:	Green powder
Lot #:	0103239911
Purity:	96.8% a.i.
Stability:	The test material was shown to be stable in the vehicle for up to 12 days at 5±3°C.
CAS # of TGAI:	14915-37-8
Structure:	

2. **Vehicle** – Aqueous 5% carboxymethylcellulose

3. **Test animals**

Species:	Rat
Strain:	Crl:CD(SD)
Age/weight at dosing:	Approximately 6-7 weeks / 144-170 g males; 137-171 g females
Source:	Charles River Laboratories (Kingston, NY)
Housing:	Individually in stainless steel wire-mesh cages
Diet:	Rodent Lab Chow #5002 (PMI Nutrition International, St. Louis MO), *ad libitum*, except during neurobehavioral testing
Water:	Reverse osmosis treated tap water (with chlorine added as a bacteriostat), *ad libitum*, except during neurobehavioral testing
Environmental conditions:	**Temperature:** 19-25°C **Humidity:** 30-70% **Air changes:** ≥10/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	At least 5 days

B. STUDY DESIGN

1. **In-life dates** - Start: 04/25/06 End: 07/27/06

2. **Animal assignment and dose rationale** – The animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. Subsets of 10 rats/sex/dose in the control and 2.25 mg/kg/day groups were allowed to recover for approximately 6 weeks after the end of the dosing period. The doses for the current study were selected based on the results of a previously conducted 28-day range-finding study (CRL Study No. AEN00007). This study is summarized and included as an Appendix to this DER.

TABLE 1. Study design [a]				
Experimental parameter	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Total number of animals/sex/group	16/sex	10/sex	10/sex	16/sex
Behavioral testing (FOB, Motor activity)	16/sex	10/sex	10/sex	16/sex
Electrophysiological testing	16/sex	10/sex	10/sex	16/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex
Recovery group	10/sex	-	-	10/sex

[a] Data were extracted from pages 27, 30, & 31 of the study report.

3. **Test substance preparation, administration, and analysis** – Test formulations were prepared daily by mixing the appropriate amount of the test material with aqueous 5% carboxymethylcellulose. Formulations were stirred continuously during dosing. Rats were dosed once daily via oral gavage for 91 consecutive days. Dose volume was adjusted daily based on individual body weights. Homogeneity (top, middle, and bottom) was determined from samples collected at the start and end of the study. In a range-finding study (CRL Study No. AEN00007), test formulations were shown to be stable for up to 12 days at 5±3°C at concentrations of 0.25 and 2.50 mg/kg/day (which bracketed the range in the current study). Actual concentration at each dose was verified at Weeks 1, 2, 4, 8, 9, and 13 in the current study.

Results

Homogeneity analysis (range as % relative standard deviation, RSD): 1.5-7.9%

Concentration analysis (range as mean % of nominal):

Concentration (mg/mL)	% of nominal
0.05	95.0-104.6
0.125	92.4-101.6
0.225	85.6-114.4

The actual concentration of the test material was outside the acceptable range in the 0.225 mg/mL formulation at Week 8 (85.6% of nominal). Analysis of backup samples could not confirm that the original results were outside the acceptable range (108.2% of nominal). Otherwise, the variation between nominal and actual dosage was acceptable. The homogeneity of both (original and backup) of the 0.225 mg/mL samples at Week 1 were outside the acceptable range of ≤5% RSD (7.9 and 5.8% RSD, respectively). Otherwise, the analytical data indicated that the mixing procedure was adequate at all other concentrations at the beginning and end of the treatment period.

4. **Statistics** – The following statistical methods were applied to the data:

Parameter	Statistical Procedures
FOB parameters using interval scales (such as grip strength tests and landing foot splay test), body weight data, food consumption	Bartlett's test was performed. If the result was not significant ($p>0.001$), then ANOVA was performed. When ANOVA was significant ($p\leq 0.05$), Dunnett's test was conducted. If Bartlett's test revealed heterogeneity of variances, the Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Motor activity	Analysis of Variance with Repeated Measures was performed. Significance ($p\leq 0.05$) was tested based on a difference between groups in the total across all measurements in a session (effect of Concentration) and on a difference between groups at specific measurement periods (interaction between Concentration and Block). If the Concentration effect was significant, Dunnett's test was performed. If the Concentration x Block interaction was significant, ANOVA test was used to evaluate the data at each measurement period, and a significant result ($p\leq 0.05$) was followed by a Dunnett's test.
FOB having graded or count scores	The Kruskal-Wallis test was performed when 75% or fewer of the scores in all the groups were tied, and when this test result was significant ($p\leq 0.05$), Dunn's test was conducted. When more than 75% of the scores in any group were tied, Fisher's Exact Test was used to compare the proportion of ties in the groups.
Clinical observation incidence data, FOB descriptive and quantal data	Contingency tables using the Variance Test for Homogeneity of the Binomial Distribution were used.
Neuropathology findings	The Fisher's Exact Test was conducted.

Statistical significance was denoted as either $p\leq 0.05$ or $p\leq 0.01$. The reviewers consider the analyses used to be appropriate.

C. METHODS / OBSERVATIONS

1. **Mortality and clinical observations** - Animals were observed twice daily (prior to daily dosing and at 60±10 minutes post-dosing) for mortality and clinical signs of toxicity. Detailed clinical observations were performed weekly throughout the pre-dosing, dosing, and post-dosing (recovery) periods. Additionally, muscle mass was evaluated in all rats once per week (prior to daily dosing). The technician "rolled" the calf muscles between his or her thumb and forefinger and evaluated the muscle mass subjectively by digital palpation.

2. **Body weight** - Animals were weighed on the days that the FOB was performed (pre-exposure and Weeks 2, 4, 8, and 13), daily during the exposure period, weekly during the recovery period, and at termination.

3. **Food consumption** – Food consumption was recorded weekly during pre-dosing, dosing, and post-dosing (recovery), and the mean absolute (g/animal/day) and relative (to body weight; g/kg/day) values were reported.

4. **Cholinesterase determination** - Cholinesterase activity was not determined.

5. **Ophthalmoscopic examinations** – The eyes of all animals were examined pre-dosing and just prior to termination.

6. **Neurobehavioral assessment**

   a. **Functional Observational Battery (FOB)** – The FOB was conducted prior to initiation of dosing and at Weeks 2, 4, 8, and 13. The technician conducting the evaluations was unaware of the dose group assignment of the animals. The open field evaluation was approximately 2 minutes in duration. The scoring criteria were not provided. The following CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*		Mobility
	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing±
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
X	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
	Feces consistency	X	Respiratory rate±	X	Urination / defecation*
		X	Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response±	X	Eye prominence*	X	Gait score*
X	Touch response±	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Vocalization	X	Backing
X	Pain response*				Time to first step
X	Pupil response*				
	Eye-blink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature±	X	Forelimb grip strength*
X	Air righting reflex±			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
X	Visual placing response		**OTHER OBSERVATIONS**		Rotarod performance

*Required parameters; ±Recommended parameters

   b. **Locomotor activity** - Locomotor activity was evaluated following the FOB (prior to initiation of dosing and at Weeks 2, 4, 8, and 13). The movements of each rat were monitored by a passive infrared sensor mounted outside a stainless steel, wire-bottom cage during a 1.5 hour period, with the number of movements and time spent in movement tabulated at 5-minute intervals. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced across test sessions and cages.

   c. **Electrophysiological testing** – Electrophysiological testing was conducted on all male and female rats on the day of final dosing (prior to administration) and on all female rats in the control and 2.25 mg/kg/day recovery groups at the end of the recovery phase of the

study. Maximum compound motor action potential (CMAP) amplitude was recorded from the flexor muscles of the hock after stimulating the sciatic nerve near the hip. The testing was conducted using a Nicolet Viking Quest system (Model #: NG030159) which was calibrated shortly before the first day of testing and shortly after the last day of testing. The technician conducting the studies was unaware of the dose group assignment of the animals. The details of the methodology were reported on pages 31-32 of the study report.

7. **Sacrifice and pathology** – All surviving animals (not assigned to the recovery groups) were sacrificed via exsanguination, after an intravenous injection of a combination of sodium heparin and sodium pentobarbital. The rats were then perfused *in situ* with neutral buffered 10% formalin, and a gross necropsy was performed. Gross lesions were retained in neutral buffered 10% formalin and shipped to Research Pathology Services, Inc. (New Britain, PA) for analysis. The calvaria were removed, and the head was immersed in the fixative. After at least 24 hours, the brains from the animals selected for neurohistological examination (5 rats/sex/dose) were excised and weighed. In rats not selected for neurohistological examination, the vertebral column was cut into segments, and the hindlimbs were removed and dissected to expose the peripheral nerves. These tissues were immersed in the fixative and, along with the head and brain, retained for possible evaluation.

   In the animals selected for evaluation of neuropathological effects (5 rats/sex/dose), tissues were further dissected to allow the evaluation of the Gasserian ganglion, spinal cord, peripheral nerves, skeletal muscles, brain, and eye as detailed in the table below. The following CHECKED (X) tissues from the control and 2.25 mg/kg/day groups were evaluated microscopically by CRL Pathology Associates (Frederick, MD).

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Forebrain		Mid-thigh
X	Center of cerebrum	X	Sciatic notch
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerve
X	Medulla oblongata	X	Tibial nerve (proximal and distal)
	SPINAL CORD	X	Peroneal nerve (fibular)
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Thoracic swelling	X	Lumbar dorsal root fibers
X	Lumbar swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
X	Gasserian ganglion	X	Cervical dorsal root fibers
X	Trigeminal nerves	X	Cervical ventral root fibers
X	Optic nerve	X	Thoracic dorsal root ganglion
X	Eyes	X	Thoracic dorsal root fibers
X	Gastrocnemius muscle	X	Thoracic ventral root fibers
	Anterior tibial muscle		

   The brain, spinal cord, and eyes (with optic nerves and retina) were trimmed, processed, embedded in paraffin, and sectioned 3 times. Each section was stained with hematoxylin and

eosin (H&E), luxol fast blue/cresyl violet, and Bielschowsky's. The skeletal muscle was trimmed, processed, embedded in paraffin, sectioned, and stained with H&E. The nerves (including the Gasserian ganglia) were embedded in plastic, and sectioned 3 times at approximately 2 μm each. Each section was stained with H&E, toluidine blue, and Bielschowsky's. Longitudinal and transverse sections were made of the cervical, thoracic, and lumbar spinal cord; and the sciatic, tibial, fibular, and sural nerves.

8. **Positive controls** - Summary data from two studies (Argus Study Nos. 012-058 and 012-104; performed in 1996 and 2002, respectively) were provided that generated positive control data and validated the procedures and observers of the performing lab to conduct the FOB and to assess motor activity effects. In the FOB portion of this study, exposure to iminodipropionitrile (**IDPN**; 250 mg/kg; 4/5 daily i.p. doses at 1 mL/kg) induced the following effects at 3 days post-dosing: (i) stereotypic behavior; (ii) slight ataxia; (iii) increased ($p<0.05$ gait abnormality; (iv) impaired air-righting reflex; (v) decreased ($p<0.05$) hindlimb grip strength; and (vi) decreased ($p<0.01$) body weight in the males. At 10 days post-dosing, additional signs observed included increased ($p<0.01$) foot splay in the males and abnormal respiratory rate in both sexes. The following effects were noted at 60 minutes post-dosing with **chlorpromazine** (6 mg/kg; single i.p. dose at 1 mL/kg): (i) unusual posture; (ii) decreased mobility in the open-field; (iii) palpebral closure (eyes half-closed); (iv) lacrimation; (v) impaired air-righting reflex; and (vi) decreased ($p<0.01$) body temperature in the males. Exposure to **d-amphetamine** (4 mg/kg; single i.p. dose at 1 mL/kg) induced stereotypic behavior and piloerection at 30 minutes post-dosing. **Carbaryl** (100 mg/kg; single oral dose at 4 mL/kg) induced the following effects at 30 minutes post-dosing: (i) unusual behavior; (ii) whole body tremors; (iii) limb twitches; (iv) unusual posture; (v) slight to moderately impaired gait (ataxic, limbs splayed/dragging, or tip-toe); (vi) lacrimation; (vii) salivation; (viii) decreased pupil response; (ix) decreased ($p<0.01$) body temperature; and (x) increased ($p<0.05$) foot splay in females. Exposure to dichlorodiphenyltrichloroethane (**DDT;** 75 mg/kg; single oral dose at 2 mL/kg) induced the following effects at 6 hours post-dosing: (i) unusual behavior; (ii) limb twitches; (iii) whole body tremors; (iv) abnormal respiration; and (v) impaired air-righting reflex. In the motor activity test, exposure to **acrylamide** (45 mg/kg; 10 daily i.p. doses at 1 mL/kg) induced decreases ($p<0.05$) in the number of moves and time spent in motion in both sexes, and **d-amphetamine** (0.75 mg/kg; 3 i.p. doses at 1 mL/kg) induced increases ($p<0.05$) in the number of moves and time spent in motion in both sexes. No positive control data that demonstrate the ability of the performing laboratory to identify neuropathological lesions were provided. However, as the tissue samples were sent to an independent pathology laboratory for evaluation, this lack of data is not considered to be a deficiency.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs** – With the exception of the signs observed in the 2.25 mg/kg/day female that was sacrificed on Day 89 (see below), no adverse treatment-related clinical signs were observed at any dose in either sex. An increased incidence of excessive salivation was noted during the dosage period in the males and females at ≥1.25 mg/kg/day. It was stated that copper omadine has been shown to be a severe irritant to the mucosal membranes and an increase in salivation following oral administration would be expected.

2. **Mortality** – At 2.25 mg/kg/day, one female (#16197) was sacrificed *in extremis* on Day 89. This animal displayed the following adverse clinical signs prior to sacrifice: (i) sparse hair coat on both forelimbs; (ii) localized alopecia on the limbs; (iii) chromodacryorrhea; (iv) ataxia; (v) limited use of both hindlimbs; (vi) hunched posture; (vii) dehydration; (vii) chromorhinorrhea; (ix) red perinasal substance; (x) emaciation; and (xi) pale extremities. Additionally, this animal displayed decreased body weight and feed consumption. FOB effects observed included decreased arousal, hunched posture, limited use of both hindlimbs, dehydration, alopecia, impaired surface righting, reduced fore- and hindlimb strength, and decreased motor activity during Week 13. Also, this animal displayed slightly reduced muscle mass from Week 4 until Week 12 when it was observed to be greatly reduced. No treatment-related gross or histopathological findings were noted. It was stated that this death was considered to be related to treatment because the clinical signs observed correlate with those previously observed with this test material. One 1.25 mg/kg/day male (#16101) was found dead on Day 48. This death was considered incidental and unrelated to treatment. All other animals survived to scheduled sacrifice.

3. **Muscle mass evaluation** – At 2.25 mg/kg/day, an increased ($p \leq 0.01$) incidence (# affected/16 vs. 0 controls) of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males and during Weeks 4 through 13 in the females (Table 2a). During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19 (Table 2b).

TABLE 2a. Incidence (# affected) of decreased muscle mass in rats treated with copper omadine via gavage for 91 days. [a]

Week	Severity	Dose (mg/kg/day)							
		0		0.5		1.25		2.25	
		Male	Female	Male	Female	Male	Female	Male	Female
1	Slightly reduced	0	0	0	0	0	0	0	0
	Normal	16	16	10	10	10	10	16	16
4	Slightly reduced	0	0	0	0	0	0	0	15**
	Normal	16	16	10	10	10	10	16	1*
5	Slightly reduced	0	0	0	0	0	0	3	16**
	Normal	16	16	10	10	10	10	13	0*
6	Slightly reduced	0	0	0	0	0	0	4**	16**
	Normal	16	16	10	10	10	10	12**	0*
9	Slightly reduced	0	0	0	0	0	0	9**	16**
	Normal	16	16	10	10	9[b]	10	7**	0*
11	Slightly reduced	0	0	0	0	0	0	12**	15**
	Normal	16	16	10	10	9	10	4**	1*
12	Greatly reduced	0	0	0	0	0	0	0	1
	Slightly reduced	0	0	0	0	0	0	5**	14**
	Normal	16	16	10	10	9	10	11**	1*
13	Greatly reduced	0	0	0	1	0	0	0	0
	Slightly reduced	0	0	0	0	0	0	6**	15**[c]
	Normal	16	16	10	9	9	10	10**	0**

[a] Data were extracted from Tables 7 and 8 on pages 136-145 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

[b] Excludes values for animal # 16101, which was found dead on Day 48.

[c] Excludes values for animal # 16197, which was sacrificed on Day 89 due to adverse clinical observations.

** Statistically significantly different from controls at $p \leq 0.01$

TABLE 2b. Incidence (# affected) of decreased muscle mass in rats treated with copper omadine via gavage for 91 days and allowed to recover for an additional 6 weeks. [a]

Week	Severity	Dose (mg/kg/day)							
		0		0.5		1.25		2.25	
		Male	Female	Male	Female	Male	Female	Male	Female [b]
14	Slightly reduced	0	0	-	-	-	-	3	9**
	Normal	10	10	-	-	-	-	7	0**
15	Slightly reduced	0	0	-	-	-	-	1	9**
	Normal	10	10	-	-	-	-	9	0**
16	Slightly reduced	0	0	-	-	-	-	1	8**
	Normal	10	10	-	-	-	-	9	1**
17	Slightly reduced	0	0	-	-	-	-	0	9**
	Normal	10	10	-	-	-	-	10	0**
19	Slightly reduced	0	0	-	-	-	-	0	7**
	Normal	10	10	-	-	-	-	10	2**

[a] Data were extracted from Tables 7 and 8 on pages 139-140 and 144-145 of the study report; n=10.

[b] Excludes values for animal # 16197, which was sacrificed on Day 89 due to adverse clinical observations.

** Statistically significantly different from controls at $p \leq 0.01$

B. <u>BODY WEIGHT AND BODY WEIGHT GAIN</u> – No adverse effects on body weight or body weight gain were noted at any dose in either sex during the dosing or recovery periods (Table 3). All statistically significant differences noted were considered incidental because they were either not adverse, not dose-dependent, and/or were transient.

TABLE 3. Mean (±SD) body weight and overall (Days 1-91) body weight gain (g) in rats treated with copper omadine via gavage for up to 91 days. [a]

Days	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
1	185.2±14.4	185.5±16.0	184.4±13.4	184.1±15.2
43	441.0±35.1	431.8±34.7	439.4±36.7	429.1±45.6
91	554.9±53.6	548.2±54.9	559.4±36.7	541.2±56.3
Overall (Days 1-91) gain	369.7±50.2	362.7±55.0	375.4±34.9	357.1±47.2
99	568.7±62.3	-	-	590.4±42.5
134	619.0±66.1	-	-	650.5±36.3
Overall (Days 91-134) gain	87.3±13.4	-	-	99.5±13.3
Overall (Days 1-134) gain	444.6±60.8	-	-	481.7±33.4
Females				
1	168.3±12.0	169.4±14.0	168.4±11.8	169.4±12.9
43	274.9±25.1	272.9±11.6	282.5±17.6	269.8±26.4
91	309.4±32.5	305.3±26.2	323.2±22.1	305.3±32.8
Overall (Days 1-91) gain	141.1±29.4	135.9±22.8	154.8±22.0	136.4±24.9
99	315.9±32.3	-	-	309.9±32.0
134	333.1±35.1	-	-	337.2±40.3
Overall (Days 91-134) gain	30.3±11.9	-	-	38.7±13.9
Overall (Days 1-134) gain	169.0±35.8	-	-	178.6±32.8

[a] Data were extracted from Tables 11-14 on pages 166-173 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

C. **FOOD CONSUMPTION** – No adverse treatment-related effects were observed on absolute or relative food consumption in either sex at any dose. The statistically significant differences noted were not considered biologically important because they were increased rather than decreased, they were minor (<12%), and the increases in absolute food consumption did not correspond with increases in relative food consumption.

D. **OPHTHALMOSCOPIC EXAMINATIONS** – No treatment-related ocular lesions were observed at any dose at the end of the dosage period.

E. **NEUROBEHAVIORAL RESULTS**

1. **FOB findings** – No treatment-related FOB effects were noted at any dose in either sex. All statistically significant findings were considered incidental because they were either (i) considered normal behavior for a given parameter, (ii) did not correspond with any unusual values in any other FOB parameter, (iii) not comparable between sexes, or (iv) not dose-dependent.

2. **Electrophysiological measurements** – In the 2.25 mg/kg/day females, the maximum amplitude of the CMAP was decreased ($p \leq 0.01$) by 28% compared to controls at the end of the dosing period (Day 91; Table 4). At the end of the recovery period (Day 134), the maximum amplitude was still decreased ($p \leq 0.05$) by 13% in the 2.25 mg/kg/day females compared to controls; however, the reduction was smaller compared to the end of the dosing period, which indicates that some recovery had occurred.

TABLE 4. Mean (±SD) electrophysiological measurements of compound motor action potential (maximum amplitude, mV) in rats treated with copper omadine via gavage for 91 days and allowed to recover for an additional 6 Weeks. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males (Day 91)	51.9±11.5	48.4±13.6	47.1±10.8	49.6±9.6
Females (Day 91)	51.6±8.9	51.9±7.8	52.2±12.0	37.1±15.6**(↓28)
Females (Day 134)	63.0±9.4	-	-	54.9±5.8*(↓13)

a Data were extracted from Tables 19 and 20 on pages 182-183 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups on Day 91, n=10 in the controls and n=9 in the 2.25 mg/kg/day group on Day 134. Percent difference from controls (calculated by reviewers) is presented parenthetically.

* Statistically significantly different from controls at $p \leq 0.05$

** Statistically significantly different from controls at $p \leq 0.01$

3. **Motor activity** – No treatment-related effects on total session motor activity (number of movements or time spent in movement) were observed at any dose in either sex (Table 5). Interval motor activity data were provided in Tables 9 and 10 on pages 146-165. Habituation was unaffected by treatment.

TABLE 5. Mean (±SD) total session motor activity in rats treated with copper omadine via gavage for up to 91 days. [a]

Observation	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
Number of movements				
Pretest	496.2±231.0	567.1±154.0	495.9±124.6	539.5±244.1
Week 2	520.2±271.5	632.4±177.2	462.4±151.5	560.7±231.5
Week 4	586.4±199.1	576.6±191.4	615.2±197.1	577.5±202.4
Week 8	575.9±293.6	634.3±231.3	511.6±127.5	740.8±263.5
Week 13	582.5±327.8	537.6±333.8	478.6±222.3	529.8±235.3
Time spent in movement (sec)				
Pretest	725.0±443.5	928.4±199.4	812.9±296.5	835.1±371.4
Week 2	755.4±399.0	1034.6±406.1	696.7±323.0	872.6±396.1
Week 4	851.8±285.1	928.4±376.3	961.2±330.8	888.8±313.7
Week 8	927.1±480.1	1071.8±372.2	963.0±273.0	1267.6±417.8
Week 13	963.2±638.0	878.6±573.2	862.0±460.0	902.2±481.9
Females				
Number of movements				
Pretest	664.8±284.9	717.7±226.3	615.2±185.7	674.4±243.5
Week 2	583.8±200.8	652.9±329.2	588.2±205.3	602.1±185.3
Week 4	690.9±199.6	600.2±201.6	645.9±264.7	709.1±179.8
Week 8	593.9±207.7	659.7±258.3	752.1±316.4	684.0±271.7
Week 13	629.3±237.0	649.7±238.6	614.3±218.2	662.1±324.7
Time spent in movement (sec)				
Pretest	984.2±412.7	1180.8±407.0	897.3±222.3	988.1±386.6
Week 2	803.9±258.6	1030.9±525.9	855.7±323.6	900.6±325.6
Week 4	934.6±308.1	931.9±306.8	1031.3±430.6	1055.5±488.5
Week 8	907.1±378.8	1195.4±453.9	1250.9±569.8	1197.1±634.5
Week 13	989.2±371.8	1100.5±374.1	975.8±523.9	1119.4±706.4

a Data were extracted from Tables 9 and 10 on pages 146-165 of the study report; n=10 for the 0.5 and 1.25 mg/kg/day groups and n=16 for the control and 2.25 mg/kg/day groups.

G. SACRIFICE AND PATHOLOGY

1. **Gross pathology** – No treatment-related gross lesions were noted in any animal.

2. **Brain weights** – No treatment-related differences in absolute or relative (to body) brain weights were observed at any dose in either sex (Table 6). The increase noted in relative (to body) brain weight in the 2.25 mg/kg/day males (↑12%; $p \leq 0.01$) was not considered to be related to treatment, but rather due to the decreased ($p \leq 0.01$) terminal body weight (↓16%) in the 5 males selected for neuropathological examination.

TABLE 6. Selected mean (±SD) absolute (g) and relative (to body, %) brain weights in rats treated with copper omadine via gavage for 91 days. [a]

Parameter	Dose (mg/kg/day)			
	0	0.5	1.25	2.25
Males				
Terminal Body (g)	572.6±38.2	545.0±27.8	568.2±41.3	483.0±40.1**(↓16)
Absolute	2.316±0.088	2.288±0.099	2.304±0.055	2.196±0.133
Relative (to body)	0.404±0.026	0.418±0.013	0.406±0.023	0.454±0.025**(↑12)
Females				
Terminal Body (g)	316.4±37.3	311.8±13.3	326.0±34.1	288.2±49.9
Absolute	2.095±0.060	2.062±0.118	2.062±0.151	2.105±0.053
Relative (to body)	0.670±0.087	0.662±0.040	0.634±0.050	0.753±0.162

[a] Data were extracted from Tables 23 and 24 on pages 186-187 of the study report; n=5.

3. **Neuropathology** – Treatment-related neuropathological lesions (# affected vs. 0 controls) were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females (Table 7). Additional findings in the muscle of these animals included minimal to mild myositis (2/5 males and 1/4 females), mild muscle fiber degeneration (2/4 females), and minimal muscle fiber necrosis (1/4 females). All other microscopic lesions noted (nerve fiber degeneration and ganglion neuron vacuolation) are commonly observed in this strain and age of rat and/or were observed in a similar or greater frequency in the controls.

TABLE 7. Neuropathological lesions in the skeletal muscle of rats treated with copper omadine via gavage for 91 days. [a]

Observation	Severity	Dose (mg/kg/day)			
		0	2.25	0	2.25
		Males		Females	
Muscle fiber atrophy	Minimal	0	1	0	0
	Mild	0	0	0	1
	Moderate	0	0	0	2
	Total	0	1	0	3*
Myositis	Minimal	0	1	0	0
	Mild	0	1	0	1
	Total	0	2	0	1
Muscle fiber degeneration	Mild	0	0	0	2
Muscle fiber necrosis	Minimal	0	0	0	1

[a] Data were extracted from Tables 1 and 2 of the pathology report on pages 988 and 994 of the study report; n=5, except for n=4 in the 2.25 mg/kg/day females.

* Statistically significantly different from controls at $p \leq 0.05$

III. DISCUSSION AND CONCLUSIONS

A. CONCLUSIONS – No compound-related effects were observed in clinical signs of toxicity, body weight, body weight gain, food consumption, ophthalmoscopic effects, FOB, motor activity, brain weights, or gross pathology.

At 2.25 mg/kg/day, the incidence of excessive salivation was increased during the dosage period in both sexes. It was stated that this effect was presumed to be due to the irritation of the oral mucosa. One female (#16197) was sacrificed *in extremis* on Day 89, and prior to sacrifice, this animal displayed adverse clinical signs that correlated with those previously observed with this test material. An increased ($p \leq 0.01$) incidence (# affected/16 vs. 0 controls) of slightly reduced hindlimb muscle mass was noted at Weeks 6 through 13 in the males (4-12) and during Weeks 4 through 13 in the females (15-16). During the recovery period, males showed signs of recovery; however, the majority of the females still displayed decreased ($p \leq 0.01$) muscle mass up to Week 19. The females also had a corresponding decrease ($p \leq 0.01$) in the average maximum amplitude in the electrophysiological measurements of CMAP (↓28%) that remained reduced, but to a lesser extent (↓13%), following the 6-week recovery period. Treatment-related neuropathological lesions (# affected vs. 0 controls) were limited to the skeletal muscle sections in the 2.25 mg/kg/day animals, with definitive muscle fiber atrophy in 1/5 (minimal) males and 3/4 (mild to moderate) females. Additional findings in the muscle of these animals included minimal to mild myositis, mild muscle fiber degeneration, and minimal muscle fiber necrosis.

At 1.25 mg/kg/day, treatment-related effects were limited to an increased incidence of excessive salivation during the dosage period in the males and females. The not-toxicological significant effects may have been due to the irritation of the oral mucosa.

The LOAEL is 2.25 mg/kg/day based on mortality, reduced hindlimb muscle mass, decreased electrophysiological measurements (females), and microscopic lesions in the skeletal muscle. The NOAEL is 1.25 mg/kg/day.

The study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OPPTS 870.6200) for a subchronic neurotoxicity study in rats.

B. STUDY DEFICIENCIES – None

III. DISCUSSION AND CONCLUSIONS

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B. STUDY DEFICIENCIES – None

APPENDIX

28-Day Dose Range-finding Study in Rats

Since this is a range-finding study, only a summary is provided to confirm the adequacy of the dose selection rationale used in the definitive subchronic oral neurotoxicity study in rats (MRID 47023701).

In a 28-day oral toxicity study (Charles River Study No. AEN00007), copper omadine (96.8% a.i., Lot No. 0103239911) in aqueous 0.5% carboxymethylcellulose was initially administered orally via gavage (10 mL/kg) once daily to 5 Sprague-Dawley rats/sex/dose at doses of 0, 0.25, 0.5, 1.25, or 2.50 mg/kg/day for 28 consecutive days. The females assigned to the 2.50 mg/kg/day group received that dose from Days 1-17; however, due to the toxicity observed, treatment was discontinued for 5 days until an improvement in their condition was observed and the dosage was reduced to 1.75 mg/kg/day. The lower dosage was administered for 14 additional days (Days 22-35).

At 1.25 mg/kg/day, reduced body weight gains were observed in both sexes.

At 2.50 mg/kg/day, the following treatment-related effects were observed: (i) adverse clinical signs (hunched posture, shuffling gait, scant feces, limited use of hindlimbs, dehydration, ungroomed coat, chromodacryorrhea, and pale extremities); (ii) mortality (sacrifice of one female rat, due to the severity of clinical signs mentioned above); (iii) decreased body weight and body weight gain; (iv) decreased food consumption; (v) reduced hindlimb muscle mass; and (vi) reduced grip strength.

The LOAEL is 1.25 mg/kg/day based upon decreased body weight gains in both sexes. The NOAEL is 0.50 mg/kg/day.

This study is classified as **Acceptable/Non-guideline.**

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

Sign-off Date : 02/15/11
DP Barcode Nos.: D375749 and D369393

TXR Nos. : 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D.
RASSB, Antimicrobial Division
EPA Secondary Reviewer: Steve Malish, Ph.D.
RASSB, Antimicrobial Division

Signature: Jonathan Chen
Date: 02/02/2011
Signature: S. J. Malish
Date: 2/7/11

DATA EVALUATION RECORD

STUDY TYPE: Metabolism – *CD rats*; OPPTS 870.7485 [§85-1)]; OECD 417.

PC CODE: 088001 **DP BARCODE:** 369393

TEST MATERIAL (PURITY): CuPT (95.2%) and [^{14}C]CuPT (carbon-14 labeled copper pyridinethione, 94.3%)

SYNONYMS: Copper-2-pyrithio-1-oxide, Copper Omadine

CITATION: Jeffcoat, A. (2007). Disposition of copper pyrithione (CuPT) in female rats: Repeated dose oral gavage pharmacokinetic studies. Research Triangle Institute. Report Number 0209593.000, July 25, 2007. MRID 47844501. Unpublished.

Jeffcoat, A. (2007). Disposition of zinc pyrithione (ZPT) in female rats: Repeated dose oral gavage and dermal pharmacokinetic studies. Research Triangle Institute. Report Number 0208173.000, July 25, 2007. MRID 47751201. Unpublished.

SPONSOR: Arch Chemicals, Inc. Cheshire, CT

EXECUTIVE SUMMARY:

In a metabolism study (MRID 47844501), [^{14}C] copper pyrithione ([^{14}C] CuPT, 95.2%, lot# 3547-058) was administered to female Crl:CD®(SD)IGS BR rats/(6 "core" and 2-3 "extra"/dose) by oral gavage at nominal dose levels of 0, 0.5, or 1.25 mg/kg bw/day for 9 days.), The pharmacokinetics, distribution, and excretion of [^{14}C] CuPT were evaluated and compared with similarly obtained data from a sister study involving zinc pyrithione (ZPT, MRID 47751201). Dose-dependent increases in measured radioactivity in the blood were seen with peak values reached 1 hour following administration of the final dose. The largest concentrations of radioactivity were detected in the liver and kidneys of rats dosed with 0.5 mg/kg or 1.25 mg/kg [^{14}C]CuPT. The majority of the administered doses was excreted in the urine (77%) and feces (8-10%) and was independent of dose. Less than 1% of the total administered dose of [^{14}C] CuPT was detected in the carcass.

A comparison of the results from the CuPT and ZPT studies indicated that total measured radioactivity and pyrithione concentrations in plasma were 1-2 times higher in animals exposed to CuPT. Pharmacokinetic parameters indicated that animals exposed to CuPT had prolonged

absorption resulting in a longer half-life of pyrithione.

This metabolism study in the rat is classified ACCEPTABLE-GUIDELINE and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

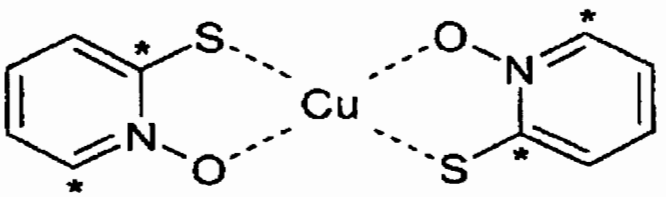
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

Radiolabelled test material:	[^{14}C]CuPT
Radiochemical purity:	94.3 ± 0.7% [determined by HPLC]
Specific activity:	9.88 mCi/mmol (31.97 mg/mCi)
Lot/batch #:	Lot # 3547-058
Non-Radiolabelled test material:	CuPT
Description:	Not provided
Lot/batch #:	Lot # A136589
Purity:	95.2% a.i. [determined by HPLC]
Contaminants:	Not provided
CAS # of TGAI:	Not provided

Structure:



* denotes site of radiolabeled carbon

2. Vehicle and/or positive control: Triethanolamine lauryl sulfate (40% aqueous solution) diluted to 0.1% (v/v) with Optima water (Fisher Scientific)

3. Test animals:

Species:	Rat
Strain:	Crl:CD®(SD)IGS BR
Age/weight at study initiation:	77-80 days/196 ± 8 g

Source:	Charles River, Raleigh, NC	
Housing:	Individual glass metabolism cages	
Diet:	Certified Purina Rodent Chow (meal form, 5002M) was provided for a period of 8 hours each day.	
Water:	Administered *ad libitum*.	
Environmental conditions:	**Temperature:**	69-76°F
	Humidity:	40-70%
	Air changes:	10/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 days	

4. Preparation of dosing solutions: CuPT and [^{14}C] CuPT were weighed and suspended in appropriate volumes of 0.1% aqueous triethanolamine lauryl sulfate. Dosing solutions were then divided into daily doses and refrigerated until needed. The non-radiolabeled formulations were used to dilute the [^{14}C] CuPT formulations to the appropriate radioactivity level.

5. Analysis of dosing solutions: [^{14}C] CuPT dose formulations were analyzed for both radiochemical and CuPT concentration. The radiochemical content was measured by liquid scintillation spectrometry (LSS) from triplicate weighed aliquots (25 ul) of each dose formulation on each day the formulation was used. The results from these daily analyses were used to calculate the dose of CuPT delivered to each animal.

The CuPT concentration in each batch of dose formulation was also confirmed by HPLC analysis (UV detection) from aliquots taken prior to dosing and on the last day the formulation was used for dosing. Derivitization of the formulations with 4-fluor-7-nitrobenzo-furazan (NBD-F) was necessary for HPLC analysis.

The radiolabel concentrations of mock dose formulations varied by 4% or less during the course of the study indicating that the formulations were stable. The formulation concentrations tested for stability were based on the original intended dose ranges for the study (0.1-2.5 mg/kg bw/day). Doses for the CuPT study were reduced based on unexpected toxicities (associated with jugular cannulization and loss of blood) observed in the rats administered 3.0 mg/kg bw/day ZPT in a companion study.

B. STUDY DESIGN AND METHODS:

1. Group arrangements: Female rats, selected because they have been shown to be more sensitive to pyrithione-induced neurotoxicity, were randomly assigned, based on body weight, to the test groups noted in Table 1. Animals in each treatment group were randomly assigned a number. Animals numbered 1-6 were designated as the core animals. Animals with higher numbers were designated as extra animals and took the place of a core animal if it was unable to complete the study.

TABLE 1: Dosing groups for pharmacokinetic studies for CuPT[a]

Test group	Target dose of [^{14}C] CuPT (mg/kg bw/day)	Actual dose of [^{14}C] CuPT (mg/kg bw/day)	Number/ sex	Remarks
Control (Group K1A)	0	0	6/F	Six core animals plus 2 extra animals started the study with 1 animal lost due to mortality. Animals were anesthetized using ketamine:xylazine (7:1; 60-80 mg/kg or to effect) prior to cannulation surgery. This set of control animals was used simultaneously for both the CuPT and companion ZPT study.
Group C-10	0.5	0.548 ± 0.008	6/F	Six core animals plus 3 extra animals started the study with 1 animal lost due to mortality. Animals were anesthetized using ketamine:xylazine (7:1; 60-80 mg/kg or to effect) prior to cannulation surgery.
Group C-30	1.25	1.40 ± 0.02	5/F	Six core animals plus 3 extra animals started the study with 4 animal lost due to mortality. Animals were anesthetized using ketamine:xylazine (7:1; 60-80 mg/kg or to effect) prior to cannulation surgery.

[a] Data obtained from page 26 of study report

2. Dosing and sample collection:

a. Pharmacokinetic studies: Oral gavage doses of [^{14}C] CuPT were administered via disposable syringes (3 mL) equipped with stainless steel gavage needles at target dose levels of 0.5 or 1.25 mg/kg bw/day to female rats (6 "core" animals/ dose group; 2-3 "extra" animals/dose) for up to 9 days. Administered doses (mg/kg weight basis) were based on the daily weights recorded for each animal. Animals were housed singly in glass metabolism cages equipped for separate collection of urine and feces. Excreta were collected over 24-hour intervals for all animals and weighed prior to analysis by liquid scintillation spectrometry (LSS) for total radioactivity. Cages were rinsed following the final excreta collection and at times of cage changes rinses (1N ethanolic sodium hydroxide with water) and the rinsings analyzed for total radioactivity. Animals were weighed daily prior to and throughout the study.

Prior to dosing, cannulas were implanted into the jugular vein of animals to facilitate the collection of serial blood samples. Blood was collected using heparinized syringes and centrifuged in instances when plasma was needed. Roughly equivalent volumes of heparinized blood from untreated rats were injected into each animal following blood sampling to prevent debilitation. Blood samples were analyzed by LSS for total radioactivity. Plasma samples, packed on dry ice and shipped overnight to a contract laboratory (PPD, Inc.), were analyzed for pyrithione.

Following sacrifice by exsanguination, the following organs were removed, weighed, and processed for LSS analysis from all "core" animals: kidney, liver, heart, stomach (including contents), small intestine (including contents), cecum (including contents), large intestine (including contents), brain, gastrocnemius muscle, sciatic and tibial nerve trunks, and spinal

cord. Carcasses were also analyzed for total radioactivity.

Animals were observed twice daily for mortality, morbidity, clinical signs of toxicity and acute distress. Functional assessments in the form of hind limb muscle tone and muscle mass were evaluated within 4 hours of sacrifice.

b. Metabolite characterization studies: No metabolite analyses were performed.

3. Statistics: Qualitative continuous data (e.g., body weights, concentration of radiolabel in various tissues, etc.) were compared between the treatment groups using either parametric ANOVA under the standard assumptions or robust regression methods. Levene's test was used to examine the homogeneity of variance and robust regression methods were used when Levene's test indicated a lack of homogeneity of variance ($p < 0.05$). In instances where Levene's Test did not indicate a lack of homogeneity, standard ANOVA methods were used.

4. Pharmacokinetic Calculations: Blood concentration–time data was analyzed using noncompartmental modeling techniques. Estimates of pharmacokinetic parameters, made after fitting a model to the blood concentration-time data, included maximum observed plasma concentration and time at which the maximum concentration was observed (C_{max} and T_{max}, respectively), terminal elimination rate constant (K_{ei}) and half-life ($t_{1/2}$), area under the plasma concentration verses time curve extrapolated to infinity ($AUC_{0-\infty}$) or until the last referenced time point (AUC_{0-t}), and mean residence time (MRT) which was calculated as the (area under the concentration x time vs. time curve $)_{0-t}/(AUC)_{0-t}$. Although no statistical analysis was provided, the results were reported as mean values with standard deviations and were considered appropriate. Pharmacokinetic analyses were conducted using VinNolin (Version 4.01; Pharsight, Palo Alto, CA) or Microsoft Excel 2002 v10.2614.2625.

II. RESULTS:

A. PHARMACOKINETIC STUDIES:

1. Preliminary experiment: No formal references to previously conducted studies with CuPT were reported. A concurrent study with ZPT, undertaken for comparative purposes, had unexpected mortalities which influenced the dose selections for the CuPT study.

2. Absorption: This study did not present absorption information.

3. Tissue distribution: Distribution of [^{14}C] CuPT in rat tissues/organs is summarized in Table 2. The greatest concentrations of radioactivity from both the 0.5 mg/kg bw/day and 1.25 mg/kg bw/day dose groups was detected in the blood, liver and kidney. Following a single dose of [^{14}C]CuPT, radioactivity concentrations in serial blood peaked 8 hours after exposure in 0.5 mg/kg bw/day rats (101 ng-eq of CuPT/g blood) and 12 hours after exposure in the 1.25 mg/kg bw/day rats (286 ng-eq of CuPT/g blood). Throughout the 9 days of dosing, concentrations of [^{14}C]CuPT in blood slowly increased peaking on day 9 at 317 ng-eq of CuPT/g blood in 0.5 mg/kg bw/day rats and 884 ng-eq of CuPT/g blood in 1.25 mg/kg bw/day rats. Following the final dose of [^{14}C]CuPT, blood concentrations of radioactivity decreased with measured mean

pseudo half-lives of 44-53 hours.

Maximum concentrations of pyrithione were measured in plasma on study day 5 (approximately 97 hours after the first dose was given) and were reported to be 31.4 ng/mL in 0.5 mg/kg/dy rats and 78.9 ng/mL in 1.25 mg/kg/dy rats.

TABLE 2: Distribution of radioactivity in rat tissues/organs after administration of [^{14}C]CuPT[a].

Tissue/organ	Mean Residual Dose in Tissues of Female Rats at Sacrifice after Repeated Dosing [ng-eq/g]	
	0.5mg/kg bw/day	1.25 mg/kg bw/day
Blood	96.8 ± 11.4	232 ± 55
Muscle (est from aliquots below)	29.5 ± 6.0	66.1 ± 12.0
Prox. Gastrocnemius muscle	30.0 ± 6.0	64.8 ± 13.3
Mid gstrocnemius muscle (A)	30.1± 5.9	65.3 ± 10.3
Mid gastrocnemius muscle	28.7 ± 5.8	69.3±14.4
Distal gastrocnemius muscle	29.2 ± 6.6	65.1 ±12.7
Brain Stem	25.4 ± 6.1	57.8 ±12.1
Cerebellum	25.2 ± 5.2	60.9 ±12.2
Rt cerebral hemisphere	27.0 ± 5.7	62.6 ±12.1
Lf cerebral hemisphere	26.3 ± 4.9	64.2 ± 15.8
Cervical spinal cord	22.6 ± 4.4	54.7 ± 8.6
Lumbar spinal cord	20.9 ± 4.7	53.6 ± 10.5
Sciatic nerves	10.8 ± 0	79.3 ± 0
Tibial nerves	65.0 ± 0	94.5 ± 0
Heart	44.4 ± 8.4	108 ± 12
Kidney	114 ± 19	270 ± 58
Liver	104 ± 18	231 ± 55
Stomach (with contents)	0.9 ± 0.3	3.0 ± 0.8
Small Intestine (with contents)	1.9 ± 0.3	5.6 ± 0.8
Cecum	1.4 ± 0.5	3.8 ± 0.7
Large Intestine (with contents)	1.2 ± 0.5	4.6 ± 1.7

[a] Data obtained from page 35 in the study report.

4. Excretion: Approximately 77% of the daily administered dose of [^{14}C] CuPT was excreted in the urine with more than half occurring within 24 hours of dosing. The cage rinse accounted

for 7.0% of the average daily dose excreted in the 0.5 mg/kg bw/day rats and 12.7% in the 1.25 mg/kg bw/day rats. Only 8-10% of the total administered radioactivity was excreted in the feces. Less than 1% of the total administered radioactive dose remained in the carcass after sacrifice. Radioactivity in expired air was not measured. Table 3 summarizes the percent of total administered radioactivity recovered in select tissues and excreta.

TABLE 3: Recovery of radioactivity in tissues and excreta of rats after repeated oral administration of C^{14}-labeled Compound CuPT [a]

	Percent of residual radioactive dose recovered	
	Repeated Oral dose of 0.5 mg/kg bw/day	**Repeated oral dose of 1.25 mg/kg bw/day**
Blood	0.15 ± 0.02	0.14 ± 0.03
Total Muscle	0.25 ± 0.06	0.22 ± 0.04
Brain Stem	0.00 ± 0	0.00 ± 0
Cerebellum	0.00 ± 0	0.00 ± 0
Spinal Cord	0.00 ± 0	0.00 ± 0
Sciatic Nerves	0.00 ± 0	0.00 ± 0
Tibial Nerves	0.00 ± 0	0.00 ± 0
Heart	0.00 ± 0	0.00 ± 0
Kidney	0.02 ± 0	0.02 ± 0
Liver	0.08 ± 0.01	0.08 ± 0.01
Stomach (with contents)	0.01± 0	0.01± 0
Small Intestine (with contents)	0.02 ± 0	0.03± 0
Cecum	0.02 ± 0.01	0.02 ± 0.01
Large Intestine (with contents)	0.01 ± 0	0.02 ± 0.01
Carcass	0.80 ± 0.17	0.72 ± 0.14
Total Residual Dose in Animal at Sacrifice	0.97 ± 0.18	0.90 ± 0.16
Residual Dose in Excreta		
Cage wash	0.77 ± 0.24	1.41 ± 0.96
Urine	76.13 ± 3.99	74.40 ± 3.72
Feces	9.84 ± 1.88	7.71 ± 1.99
Total Excreted Dose	86.74 *	83.52 *
Total Recovered Dose	Not reported	Not reported

[a] Data obtained from pages 128, 132, 136, 148, 152, 156 in the study report.
* Calculated by reviewer

5. Pharmacokinetic Parameters: Maximum concentrations of pyrithione in plasma (C_{max}) were reached within 0.5-2 hours following oral administration of [^{14}C] CuPT. Mean elimination half-lives ($T_{1/2}$) of [^{14}C] CuPT were similar in 0.5 mg/kg bw/day rats on study day 1 (8.0 hours) and on study day 9 (7.9 hours). In comparison, $T_{1/2}$ values in the 1.25 mg/kg bw/day rats showed more variation with values of 5.8 hours on study day 1 and 10.1 hours on study day 9. These

values were considerably lower than the half-lives of 44-53 hours reported for total radioactivity in blood. C_{max} and systemic exposures (expressed as AUC) both showed dose related increases following dosing of [^{14}C] CuPT on days 1 and 9. C_{max} and AUC ratios (day 9 / day 1) were less than 1.5 for both the 0.5 mg/kg bw/day and 1.25 mg/kg bw/day rats indicating accumulation of pyrithione was minimal following repeated oral exposure to [^{14}C] CuPT for 9 days.

Table 4. Summary of Pharmacokinetic Parameters[a,b]

Dose (mg/kg bw/day)	Study Day	T max (h)	Mean Cmax (ng/mL)	Mean K_{ei} (h^{-1})	Mean T1/2 (h)	AUC 0-24 hr x ng/mL	AUC 0-∞ (h x ng/nL)	MRT (h)	Cmax ratio	AUC Ratio (0-24 h)
0.5	1	0.92 ± 0.58	32.9 ± 14.7	0.11 ± 0.04	8.0 ± 4.9	135 ± 13.1	146 ± 18	5.42 ± 1.28	1.0 ± 0.5	1.1 ± 0.2
0.5	9	1.12 ± 0.48	29.6 ± 11.3	0.09 ± 0.02	7.9 ± 2.2	152 ± 16.1	151 ± 17	5.82 ± 1.42		
1.25	1	1.33 ± 0.52	70.1 ± 23.9	0.12 ± 0.02	5.8 ± 1.1	352 ± 52	367 ± 49	5.68 ± 1.08	0.8 ± 0.3	1.4 ± 0.4
1.25	9	1.59 ± 0.56	50.2 ± 5.02	0.09 ± 0.06	10.1 ± 4.8	506 ± 133	506 ± 140	14.0 ± 9.5		

[a] Data obtained from page 32 of study report
[b] n=6
t_{max} = time at which C_{max} was observed.
C_{max} = observed maximum concentration of CuPT in plasma
K_{el} = terminal elimination rate constanrt
$t_{1/2}$ = elimination half-life
AUC = area under the plasma concentration vs. time curve
MRT = mean residence time (0-24 h)

6. **Functional Assessments:** Muscle mass and muscle tone decreased with increasing dose of [^{14}C] CuPT. While assessments of muscle mass and muscle tone were similar in the control rats and the 0.5 mg/kg bw/day rats, significant reductions in muscle mass and muscle tone were observed in the 1.25 mg/kg bw/day rats. It should be noted that these assessments were reported as subjective alphabetical ratings rather than measured, numerical parameters.

7. **Body Weights:** The 0.5 mg/kg bw/day rats showed an increase in body weight compared with the control rats while the 1.25 mg/kg bw/day rats showed a slight decrease in body weight on days 6-12 and an increase on day 13 following a reduction in the number of animals in the group from 6 to 5 due to mortality. No statistical analysis was conducted with this data.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

B. REVIEWER COMMENTS:

The purpose of this study was to evaluate the disposition of CuPT in rats following repeated oral

exposure for comparison with the disposition profile established for zinc pyrithione (ZPT) in a similar study (MRID 47751201). Following repeated oral dosing with [^{14}C]CuPT for 9 days at nominal doses of 0.5 mg/kg bw/day or 1.25 mg/kg bw/day, the majority of the administered dose was excreted in the urine (77%) and feces (8-10%). The amounts excreted were determined to be independent of dose level. Less than 1% of the total administered dose of [^{14}C]CuPT was detected in the carcass.

Comparison of the pharmacokinetic parameters of pyrithione obtained from the studies with CuPT and ZPT is possible since both studies used identical doses of 1.25 mg/kg bw/day and the molecular weights of the substances are similar. T_{max} for pyrithione derived from CuPT was approximately three times longer than when derived from ZPT while C_{max} values for CuPT are less than half those from ZPT. The 0-24 h AUC for pyrithione derived from CuPT is almost twice that for pyrithione derived from ZPT indicating that absorption of pyrithione derived from CuPT is more prolonged that from ZPT. Urinary and fecal excretion results were similar for both studies.

C. STUDY DEFICIENCIES:

Minor Un-upgradable Deficiency:

- Recovery of radioactivity in expired air was not measured nor was a statement included stating that it was not relevant.
- Less than 90% of administered radioactive dose was recovered.

Minor Upgradable Deficiency: (upgradeable with appropriate data)

- Description of test material was not provided.
- Documentation for Tier 2 study stating that study was conducted according to mutual agreement between registrant and the Agency is missing.
- Unclear procedural explanation regarding use of 3 mL syringe to deliver 4-5 mL dose.
- Supporting information regarding ZPT study results not included.

D. STUDY CLASSIFICATION: This metabolism study in the rat is classified ACCEPTABLE-GUIDELINE and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat.

Sign-off Date : 02/15/11
DP Barcode Nos.: D375749 and D369393

TXR No. : 1,003,204

EPA Reviewer: Jonathan Chen, Ph.D. **Signature:** Jonathan Chen
RASSB, Antimicrobials Division (7510P) **Date:** 02/15/2011
Secondary Review: Tim McMahon, Ph.D. **Signature:**
Senior Scientist, Antimicrobials Division (7510P) **Date:** 2/15/11

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Metabolism – Rat (non-guideline; registrant submitted protocol)

PC CODE: 088001 **DP BARCODE:** 375749

TEST MATERIAL (PURITY): Copper Omadine (97% a.i.)

SYNONYMS: Copper pyrithione

CITATION: Thomas, J. A. (2010) Inhalation of ^{14}C-Copper Omadine. WIL Research Laboratories, LLC, Ashland, OH. Report number: WIL-544012, February 19, 2010. MRID 48006401. Unpublished.

SPONSOR: Arch Chemicals, Inc., 350 Knotter Dr., Cheshire, CT, 06410 USA

EXECUTIVE SUMMARY:

In a metabolism study (MRID 48006401), [pyridinyl-2,6-^{14}C] copper omadine (>99% radiochemical purity, lot# 3620149) was administered to two groups of jugular vein-cannulated, female Sprague-Dawley (Crl:CD) rats (15 rats/group) by inhalation as an aerosolize dust at target concentrations of 0.5 and 1.5 mg/m^3. All animals received a single, 6-hour exposure to the ^{14}C-test substance using a dynamic nose-only inhalation exposure system. Each dose group was divided into three subgroups (5 rats/subgroup), which received daily 6-hour exposures to non-labeled copper omadine (97% ai., lot# 0103239911) at target concentrations of 0.5 and 1.5 mg/m^3 for either 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. The gravimetrically determined concentrations for the ^{14}C-test substance were 0.53 and 1.49 mg/m^3. Based on the 6-hour exposure, these concentrations were calculated to be equivalent to doses of 0.153 and 0.428 mg/kg, assuming a respiration rate of 0.8 L/minute/kg body weight for the rats. The average concentrations of non-labeled test substance during the 4- and 9-day pretreatments were respectively 0.61 and 0.55 mg/m^3 for the low-dose group and 1.60 and 1.59 mg/m^3 for the high-dose group.

Following exposure to the [^{14}C] copper omadine, blood samples were collected from each animal at 0.25, 0.5, 1.0 and 24 hours post-exposure, and urine, feces and cage wash samples were collected from 0-24 hours. Animals were sacrificed at 24 hours post-exposure, and the carcasses were washed and rinsed to assess external exposure. Selected tissues and the residual carcass were then collected for radioassay.

Maximum concentrations of radioactivity were observed in the plasma at 0.5 or 1 hour post-exposure. Maximum plasma concentrations were similar for the low-dose subgroups (0.043-0.060 μg/g) regardless of the number of prior exposures. However, for the high-dose group, the maximum plasma concentrations decreased from 0.185 to 0.100 μg/g as the number of exposures to non-labeled test substance increased.

The radioactive dose was almost completely absorbed regardless of the number of prior exposures. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥98.9% of the administered dose (AD) for both dose groups. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for <0.5% of the AD. Regardless of the number of previous exposures, urinary excretion accounted for 62.7-67.8% of the AD for the low-dose group and 59.5-64.3% of the AD for the high-dose group, with the greatest amount being excreted in the urine within 12 hours of exposure. Fecal elimination was a minor route of excretion, accounting for ≤4.8% of the AD in all subgroups. Radioactivity remaining in the carcass and tissues at 24 hours post-exposure accounted for 25.8-33.5% of the AD.

For both dose groups, radioactivity was detectable in all tissues at 24 hours post-exposure, and the relative distribution of the radioactive dose was similar among tissues. Concentrations of ^{14}C-residues were highest in the liver (low dose – 0.150-0.159 μg/g; high dose – 0.194-0.401 μg/g) and lowest in brain, spleen and lungs (low dose – 0.017-0.027 μg/g; high dose – 0.062-0.128 g/g). For the low-dose subgroups, the concentration of radioactivity in a given tissue was similar regardless of the number of previous exposures. However, for the high-dose group, concentrations of ^{14}C-residues in tissues decreased significantly ($p<0.05$) as the number of prior exposures increased. For both dose groups, the liver (2.6-5.0% AD) and carcasses (20.2-28.9% AD) accounted for the majority of the dose remaining in the body at 24 hours post-exposure.

This metabolism study in the rat is classified **acceptable/non-guideline.** The study was conducted according to a protocol submitted by the registrant and reviewed by the Agency for the purpose of examining absorption, disposition, and excretion of copper pyrithione by the inhalation route and to compare these results to a similar study conducted with zinc pyrithione (MRID 48006402) for bridging purposes. As ~ 62.7-67.8% of the recovered dose was excreted in urine, it would be useful to characterize the nature of the residue in urine for comparative purposes to the existing metabolism/kinetic data on oral exposure to zinc pyrithione.

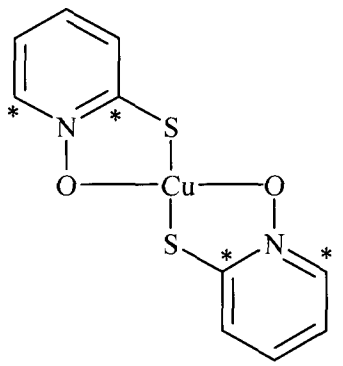
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with EPA (40 CFR Part 160), and OCED [C(97) 186/Final)] Good Laboratory Practices and the standard operating procedures of WIL Research Laboratories, LLC. The only notable protocol deviation in the study involved the loss of the gross necropsy report for one animal in Group 1B (female #52153) that died after initiation of treatment with the non-label test substance. This deviation did not have an adverse impact on the study findings.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	[Pyridinyl-2,6-^{14}C] Copper omadine
Radiochemical purity:	99.75% (determined by HPLC)
Specific activity:	20.1 mCi/mmol (63.64 ΦCi/mg)
Lot/batch #:	3620149
Non-Radiolabeled test material:	Copper omadine (copper pyrithione)
Description:	(e.g. technical, green powder, stable at room temperature)
Lot/batch #:	0103239911
Purity:	97 ± 0.2 % a.i. (determined by HPLC)
Contaminants:	NA
CAS#:	14915-37-8
Molecular weight	315.85
Structure:	



* indicates position of ^{14}C-label

2. Vehicle and/or positive control: Not applicable.

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley (Crl:CD[SD]); jugular vein-cannulated females	
Age/weight at study initiation:	147-195 g/ 6-7 weeks	
Source:	Group 1 – Charles River Laboratories (Portage, MI) Group 2 – Charles River Laboratories (Raleigh, NC)	
Housing:	Individually in suspended wire mesh cages during non-exposure periods. Following exposure to the ^{14}C-labeled test substance, rats were housed individually in plastic metabolism cages suitable for the separate collection of urine and feces.	
Diet:	Certified Rodent LabDiet® 5002; PMI Nutrition International, LLC; *ad libitum*	
Water:	Reverse-osmosis treated water, *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 3EC
	Humidity:	50 ± 20%
	Air changes:	10 /hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	≥5 days	

4. Preparation of test substance used for exposure: The animals were exposed directly to the undiluted non-labeled and ^{14}C-labeled copper omadine as an aerosolized dust.

B. STUDY DESIGN AND METHODS:

The objective of the study was to determine the absorption, distribution, and excretion profile of [^{14}C] copper omadine® in female rats following a single nose only inhalation exposure which was conducted after either 0, 4, or 9 prior nose-only inhalation exposures to non-labeled copper omadine®. The metabolism of [^{14}C] copper omadine was evaluated at target concentrations of 0.5 and 1.5 mg/m^3.

1. **Study dates:** Start: August 1, 2009; End: October 15, 2009

2. **Group arrangements:** Two groups of jugular vein-cannulated female rats (15 rats/group) were exposed to [^{14}C] copper omadine (>99% radiochemical purity) as a single, nose-only inhalation for 6 hours at target concentrations of 0.5 and 1.5 mg/m^3. Each group was subdivided into three subgroups (5 rats/subgroup), which received daily 6-hour exposures of non-labeled copper omadine (97% ai.) for either 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. Animals were assigned randomly assigned to the test groups noted in Table 1.

   No rationale was provided as to why the 0.5 and 1.5 mg/m^3 concentrations were selected for the metabolism study. However, this study was conducted concurrently with a 4-week inhalation toxicity study on rats (MRID 48006403) which utilized target exposure levels of 0.5, 1.5 and 5.0 mg/m^3.

TABLE 1: Dose groups for [^{14}C] Copper Omadine Metabolism studies using Inhalation Exposure [a]

Test group	Subgroup	Exposure concentration (mg/m^3)		Number of exposure[b]	Number females	Remarks
		Target	Actual [a]			
Group 1	A	0.5	0.53 ± 0.27	10	5	Blood was sampled from each rat at 0.25, 0.5, 1 and 24 hours post-^{14}C-dose. Urine and feces were collected from 0-24 hours post-exposure. Animals were sacrificed at 24 hours post-exposure, and selected tissues were collected for radioassay.
	B	0.5	0.53 ± 0.27	5	3 [c]	
	C	0.5	0.53 ± 0.27	1	4 [c]	
Group 2	A	1.5	1.49 ± 0.19	10	5	
	B	1.5	1.49 ± 0.19	5	5	
	C	1.5	1.49 ± 0.19	1	5	

[a] Data obtained from page 36 in the study report.

[b] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled copper omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. Note that the schedule for exposure was 5 days/week; therefore, the rats receiving 10 doses were treated over a 2-week period.

[c] One or two animals from Group 1 died during exposure.

3. **Dosing:** The non-labeled test substance was administered as a dust aerosol via nose-only inhalation for 6 hours per day for either 0, 4 or 9 days, followed by a single exposure with the ^{14}C-copper omadine as an aerosolized dust via nose-only inhalation for 6 hours. The exposure system is described in the following section.

Prior to the initial exposure, the animals were acclimated to restraint in nose-only exposure restraint tubes by increasing the restraint time over the 5 day acclimation period, from 1 hour on the first day to 6 hours by the fifth day. Following each restraint period, animals were observed for clinical signs of injury or stress. Animals were held in the restraint tubes for 25 to 63 minutes prior to the initiation of exposure. Animals were weighed prior to exposure to the non-labeled test substance and again prior to exposure to the ^{14}C-test substance.

For each exposure, the animals were placed into exposure restraint tubes, placed on the nose-only system, exposed for 6 hours, and then returned to their home cages. Animals were housed individually in plastic mesh cages during non-exposure hours. Food and water were withheld during the exposure period. Immediately following the 6-hour exposure to the [^{14}C] copper omadine, the rats were transferred to plastic metabolism cages.

4. **Generation of the test atmosphere / chamber description:** A diagram of the test atmosphere generation system and exposure chamber used for exposure is included in Appendix I.

Exposures to the non-labeled copper omadine were conducted using a 14.1-L conventional dynamic nose-only exposure system (designed and fabricated by WIL Research Laboratories, LLC) with synthetic rubber grommets in exposure ports to engage animal holding tubes. Exposure to the [^{14}C] copper omadine were conducted using a similar 7.9-L nose-only exposure system.

Air supplied to the nose-only system was provided from a dry compressed air source. All test atmosphere exhaust passed through the facility exhaust system, which consisted of charcoal- and HEPA-filtration. Exposure chamber temperature, relative humidity, and chamber ventilation rate were continually monitored and manually recorded at approximately 60-minute intervals during the exposure. The mean temperature and relative humidity were set for 19-25°C and 30-70%, respectively. During the exposures, actual mean temperatures were 21-22°C and the relative humidity was 42-54%.

For generation of the dust aerosol atmosphere, the test substance was metered and aerosolized using a Wright Dust Feeder (model WDF-II, BGI, Inc., Waltham, MN) and speed controller (model F-352-BM, Electro-Craft Servo Products, Robbins & Myers, Inc., Hopkins, MN), which delivered the test substance aerosol at a constant rate into a 4.9-L chromatography jar. The WDF was equipped with a 1.3-cm^3 stainless steel cup, which was packed with test substance. Dry compressed air was supplied to the WDF at a rate of 13.2-14.1 LPM to deliver test substance aerosol to the chromatography jar where large particles were removed prior to entering the nose-only exposure system. Humidified air was also added to the chromatography jar at a rate of 35.3 LPM using a regulator and flowmeter. The resulting aerosol from the chromatography jar was then delivered to the nose-only exposure system through ¾-inch ID anti-static tubing at a rate of ca. 49 LPM. The actual flow rates for the exposures were 49.0-57.8 LPM.

Particle size determination – Aerosol particle size determinations were conducted at least once for each sub-group using a 7-stage stainless steel cascade impactor. Pre-weighed, 22-mm stainless steel discs coated with a collection solution were used as the collection

substrates for each stage and a 25-mm glass-fiber filter was used in the tail cup. During the non-labeled test substance exposures, samples were collected at approximately 1.8 LPM for 720 and 360 minutes for the 0.5 and 1.5 mg/m^3 exposures, respectively. For the labeled test substance exposures, samples were collected at approximately 2 LPM for 393 and 402 minutes for the 0.5 and 1.5 mg/m^3 exposures, respectively. The substrates were re-weighed and the particle size was calculated based on the impactor stage cut-offs, with the aerosol size being expressed in terms of the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). The particle size determinations for the exposures to non-labeled test substance are listed below:

Subgroup	Concentration (mg/m^3)	# exposures	# samples	Mean MMAD (µm)	Mean GSD (µm)
1A	0.5	9	3	2.1	2.11
1B	0.5	4	2	2.3	2.25
2A	1.5	9	3	2.1	2.07
2B	1.5	4	2	2.3	2.13

For the single 6-hour exposure to the ^{14}C-labeled test substance, which was conducted on the same day for the subgroups in a given dose group, the MMAD and GSD were respectively 2.8 and 2.59 µm for the 0.5 mg/m^3 dose group and 3.0 and 2.35 µm for the 1.5 mg/m^3 dose group.

Test atmosphere concentrations – Actual exposure concentrations were determined using standard gravimetric methods for exposure to both the non-labeled and ^{14}C-labeled test substances. Samples were collected on pre-weighed, 25-mm glass-fiber filters held in open-faced filter holders positioned in the animal breathing zones of the nose-only exposure systems. Following sample collection, the filter was re-weighed and the concentration calculated as the filter weight difference divided by the sample volume. For the exposures to the non-labeled test substance, 2-3 samples were collected for each daily exposure and the mean concentration was reported for each day. For the low-dose group, the average daily concentration (±SD) was 0.55 ± 0.129 mg/m^3 for the 9-day exposure subgroup and 0.61 ± 0.161 mg/m^3 for the 4-day exposure subgroup. For the high-dose group, the average daily concentration (±SD) was 1.59 ± 0.117 mg/m^3 for the 9-day exposure subgroup and 1.60 ± 0.082 mg/m^3 for the 4-day exposure subgroup.

On the day of exposure to the ^{14}C-labeled test substance, 7 filter samples were collected for each dose group. The average concentration (±SD) was 0.53 ± 0.268 mg/m^3 for the low-dose group and 1.49 ± 0.19 mg/m^3 for the high-dose group. Assuming a respiratory rate of 0.8 L/minute/kg body weight for the rats, the average daily dose for the 6-hour exposure was equivalent 0.153 mg/kg for the low-dose group and 0.428 mg/kg for the high-dose group.

Exposure concentrations for the ^{14}C-label test substance were also determined radiometrically. The filters were extracted by vortexing and sonication with methanol, and the resulting extracts were radioassayed in duplicate by liquid scintillation counting (LSC). Based on these radioassays, the average exposure concentration (±SD) was 0.37 ± 0.18 mg/m^3 for the low-dose group and 1.07 ± 0.27 mg/m^3 for the high-dose group. The study author attributed the lower estimates of exposure concentrations from the radioassay to be due to the inability to fully extract the ^{14}C-copper omadine from the filters. However, no

recovery data were provided using ^{14}C-fortified filter samples. For purposes of this report, the gravimetrically determined dose levels will be utilized for all discussions.

5. **Clinical observations:** All animals were observed for mortality and morbidity twice daily throughout the study.

6. **Sample collection:** Following the 6-hour inhalation exposure to the [^{14}C] copper omadine, blood samples were collected from each animal at 0.25, 0.5 and 1 hour post-exposure via the jugular vein cannula or from the retro-orbital sinus. For collections from the retro-orbital sinus, animals were anesthetized with inhaled isoflurane. Final blood samples were also collected after sacrifice (24-hour) from the vena cava. Blood samples were collected into tubes containing an anticoagulant and were centrifuged to obtain plasma samples for radioassay. Samples of urine and feces were collected over ice at the following intervals: 0-6, 6-12 and 12-24 hours post-exposure. At each collection interval, cage surfaces were rinsed with deionized water, and the cage wash samples were retained for separate analysis. All rats were euthanized at 24 hours post-exposure by the inhalation of carbon dioxide. Each carcass was washed twice with Exodontia sponges wetted with a 1:50 (v/v) solution of Ivory liquid soap:deionized water, then rinsed twice with Exodontia sponges wetted with deionized water, and finally wiped dry with two Exodontia sponges. After drying, the final blood sample was collected from each animal along with the following tissues: lung, liver, kidney, spleen, brain, stomach, and gastrointestinal (GI) tract. The remaining carcass was weighed and retained. All samples were held on wet ice until storage at -20°C.

7. **Radioassay:** Subsamples of plasma, urine, cage wash, and cage rinse were radioassayed in duplicate for total radioactivity directly by LSC without further processing. Tissue and carcass samples were chopped and homogenized and then radioassayed in duplicate by combustion/LSC. Fecal samples were homogenized with distilled water (2:1) and radioassayed in duplicate by combustion/LSC. Filter samples from the nose-only exposure system were extracted by vortexing and sonication with methanol. The resulting methanol extract was then radioassayed in duplicate by LSC. The wash sponges were digested in 50% sulfuric acid:methanol (50:4, v/v), diluted with water and then radioassayed directly by LSC.

   The recovery of radioactivity from the various sample types was validated by fortifying samples collected from control animals with known amounts of [^{14}C]copper omadine. The recovery of radioactivity from duplicate, fortified samples of urine, feces, tissues, plasma, cage washes, and sponge digests ranged from 95.3 to 100%. The calculated limits of quantitation (LOQ) for the radioassays ranged from 0.0003 µg/g for the cage washes to 0.0035 µg/g for plasma.

8. **Metabolite characterization studies:** Although samples of urine and feces were collected in this study, no analyses were performed to identify or characterize the nature of the ^{14}C-resdiues in excreta.

9. **Statistics:** Means with standard deviations (SD) were calculated for the various parameters measured for each dose subgroup (i.e. Concentrations of radioactivity in tissues, % AD in tissues, carcass and excreta). Data from individual animals were evaluated as possible outliers using the Dixon Q-tests (Dean and Dixon, 1951). Based on this test, data from a

single rat from the 1 exposure subgroups of both dose groups were excluded from the calculation of mean radioactivity in plasma, tissues, and excreta. A two-sample t-test assuming unequal variances was performed to identify any significant differences ($p<0.05$) in concentrations of radioactivity in tissues between the subgroups.

II. RESULTS:

A. PHARMACOKINETIC STUDIES:

1. **Clinical Observations:** A single female was found dead following 2 days of exposure to non-labeled copper omadine at 0.5 mg/m^3. Although a complete necropsy was reportedly performed, the information was not included in the study. Three additional females were also found dead prior to any exposure to the test substance. As the majority of deaths occurred in non-exposed animals, the study author attributed the animal deaths to factors associated with the presence of an in-dwelling venous cannula, and the stress from confinement in the nose-only exposure.

1. **Absorption:** For both the low- and high-dose groups, the radioactive dose was almost completely absorbed regardless of the number of pre-treatment days. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥98.9% of the AD for both dose groups (See Tables 4 and 5). Even if radioactivity associated with the lungs is not considered to be absorbed, the levels of absorption would still be >98% of the AD, as the lungs accounted for only 0.1% of the AD in the low-dose group and 0.1-0.2% of the AD in the high-dose group. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for ≤1.1% of the AD.

2. **Plasma kinetics:** The maximum observed concentration of radioactivity in plasma occurred at 0.5 or 1 hour post-exposure regardless of the test substance exposure concentration or the number of previous exposures (Table 2). The maximum concentrations of radioactivity were 0.043-0.060 µg/g for the low-dose group and 0.100-0.185 µg/g for the high-dose group. By 24 hours post-exposure, plasma concentrations decreased to 0.021-0.028 µg/g for the low-dose group and 0.064-0.133 µg/g for the high-dose group.

   The increase in plasma concentrations for the high-dose group generally reflected the 3-fold increase in the dose level. Compared to the low-dose group, the concentrations of plasma radioactivity for the high-dose subgroups were generally 5-fold higher after 1 exposure, 2.8-fold higher after 5 exposures, and 2.2-fold higher after 10 exposures.

   There was no apparent correlation between plasma concentration and the number of previous exposures for the 0.5 mg/m^3 exposure group. However, for 1.5 mg/m^3 dose group, plasma concentrations of radioactivity appeared to decrease at each sampling interval with an increasing number of exposures.

TABLE 2: Distribution of radioactivity in tissues/organs of female rats 24 hours following a single 6-hour inhalation exposure of [^{14}C] Copper Omadine at 0.5 or 1.5 mg/m^3 [a]

Sampling time (hr)	Mean concentration of radioactivity in plasma (µg equivalents/g)					
	0.5 mg/m^3			1.5 mg/m^3		
	1 exposure	5 exposures	10 exposures	1 exposure	5 exposures	10 exposures
0.25	0.035 ± 0.011	0.042 ± 0.017	0.035 ± 0.013	0.165 ± 0.048	0.112 ± 0.042	0.080 ± 0.009
0.5	0.037 ± 0.012	0.051 ± 0.023	0.045 ± 0.013	0.171 ± 0.043	0.142 ± 0.054	0.096 ± 0.010
1.0	0.043 ± 0.012	0.060 ± 0.028	0.052 ± 0.022	0.185 ± 0.044	0.141 ± 0.044	0.100 ± 0.008
24	0.021 ± 0.005	0.027 ± 0.012	0.028 ± 0.010	0.133 ± 0.042	0.096 ± 0.046	0.064 ± 0.016
C_{max} (µg/g)	0.043	0.060	0.052	0.185	0.142	0.100
T_{max} (hr)	1	1	1	1	0.5	1

[a] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled copper omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to ^{14}C-exposure. Data are the average of 3-5 rats/subgroup and were obtained from page 37 in the study report.

3. **Tissue distribution:** Concentrations of radioactivity in tissues at 24 hours post-exposure are summarized in Table 3. Following the 6-hour exposure to [^{14}C] copper omadine at 0.5 mg/m^3, the mean concentrations of radioactivity at 24 hours post-exposure were highest in liver (0.150-0.159 µg/g) and kidneys (0.053-0.071 µg/g) for all subgroups and lowest in the brain, spleen and lungs (0.017-0.027 µg/g). The concentration of radioactivity in the remaining tissues and carcasses were similar, ranging from 0.024 to 0.051 µg/g. The concentration of radioactivity in any given tissue was similar regardless of the number of previous exposures. The liver accounted for 3.8-5.0% of the AD, the residual carcasses accounted for 20.2-24.9% of the AD, and the remaining tissues each accounted for ≤1% of the AD.

   Following the 6-hour exposure to [^{14}C] copper omadine at 1.5 mg/m^3, the relative distribution of radioactivity among tissues was similar to the low-dose group; however, the high-dose group showed decreases in the tissue concentrations with an increasing number of prior exposures. For all high-dose subgroups, the mean concentrations of radioactivity at 24 hours post-exposure were highest in liver (0.194-0.401 µg/g) and lowest in the brain, spleen and lung (0.052-0.128 µg/g). The concentration of radioactivity in the remaining tissues and carcasses were similar within each subgroup, ranging from 0.178-0.233 µg/g for 1 exposure, 0.127-0.181 µg/g for 5 exposures, and 0.069-0.118 µg/g for 10 exposures. The decreases in tissue concentrations of radioactivity associated with the increased number of exposures were statistically significant ($p<0.05$) for the high-dose group. Compared to a single exposure, the concentrations in tissues and the carcass generally declined by 0.7x with 5 exposures, and by 0.5x with 10 exposures. As in the low-dose group, the liver (2.6-3.3% AD) and carcass (23.9-28.9% AD) accounted for majority of the dose remaining in the body at 24 hours post-exposure.

TABLE 3: Distribution of radioactivity in tissues/organs of female rats 24 hours following a single 6-hour inhalation exposure of [^{14}C] Copper Omadine at 0.5 or 1.5 mg/m^3 [a]

Tissue/organ [b]	Concentration of radioactivity (µg equivalents/g)					
	0.5 mg/m^3			1.5 mg/m^3		
	1 exposure	5 exposures	10 exposures	1 exposure	5 exposures	10 exposures
Brain	0.017 ± 0.004	0.022 ± 0.009	0.022 ± 0.008	0.107 ± 0.036	0.076 ± 0.035	0.052 ± 0.012
Lung	0.021 ± 0.005	0.025 ± 0.011	0.027 ± 0.010	0.128 ± 0.042	0.090 ± 0.039	0.062 ± 0.014
Stomach	0.024 ± 0.010	0.028 ± 0.013	0.032 ± 0.012	0.178 ± 0.063	0.127 ± 0.093	0.069 ± 0.018
Kidney	0.053 ± 0.013	0.064 ± 0.022	0.071 ± 0.026	0.233 ± 0.079	0.181 ± 0.069	0.118 ± 0.029
Liver	0.150 ± 0.046	0.158 ± 0.045	0.159 ± 0.052	0.401 ± 0.159	0.325 ± 0.099	0.194 ± 0.055
Spleen	0.017 ± 0.005	0.022 ± 0.010	0.022 ± 0.007	0.114 ± 0.038	0.082 ± 0.038	0.055 ± 0.013
GI tract	0.033 ± 0.014	0.034 ± 0.016	0.039 ± 0.015	0.215 ± 0.080	0.128 ± 0.059	0.086 ± 0.031
Carcass	0.035 ± 0.014	0.037 ± 0.019	0.051 ± 0.025	0.233 ± 0.097	0.141 ± 0.079	0.092 ± 0.022

[a] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled copper omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to ^{14}C-exposure. Data are the average of 3-5 rats/subgroup and were obtained from pages 38 and 40 in the study report.

[b] All tissue and organ samples were collected at 24 hours post-^{14}C-exposure.

4. **Excretion:** The recovery and excretion of radioactivity following a 6-hour inhalation exposure to [^{14}C] copper omadine at target concentrations of 0.5 and 1.5 mg/m^3 are presented in Tables 4 and 5, respectively. Although the overall recovery of the low- and high-dose groups averaged 96.1 and 100% of the AD, respectively, the overall recovery of the calculated dose varied considerably between the various subgroups within each dose group. The recoveries ranged from 76.8 to 116.8% of the AD for the low-dose subgroups and from 68.7 to 141.0% of the AD for high-dose subgroups. The study author did not provide an explanation for the observed variability. However, the high degree of variability between animals is likely due to the differential respiration rates for the individual rats, resulting in a higher degree of variability in the achieved doses. The calculated dose for each animal assumed a standard respiration rate of 0.8 L/minute/kg body weight. In order to compare values across the subgroups, the amount of radioactivity (%AD) associated with each fraction was normalized such that the overall recovery for each subgroup was 100%.

   When normalized to account for the overall recovery in each subgroup, the pattern of excretion was similar for both dose groups. Regardless of the number of previous exposures, urinary excretion accounted for 62.7-67.8% of the AD for the low-dose group and 59.5-64.3% of the AD for the high-dose group by 24 hours post-exposure, and for both dose groups, the 0-6 hour urine fraction accounted for the largest fraction of radioactivity in the urine (low-dose – 31.1-39.7% AD; high-dose – 25.1-31.8% AD). By 24 hours post-exposure, fecal excretion accounted for ≤4.8% of the AD for all subgroups, and radioactivity remaining in the carcass and tissues accounted for 25.8-31.7% of the AD for the low-dose group and 28.7-33.5% of the AD for the high-dose group.

TABLE 4: Recovery of radioactivity in tissues and excreta of female rats following a single 6-hour inhalation exposure of [^{14}C] Copper Omadine at 0.5 mg/m^3 [a]

Sample/interval	Percent of administered radioactive dose [b]		
	1 Exposure	5 Exposures	10 Exposures
Expired air	NS	NS	NS
Urine 0-6 hr	23.91 ± 3.58 (31.1)	37.86 ± 13.57 (39.7)	44.59 ± 13.99 (38.2)
6-12 hr	12.98 ± 2.83 (16.9)	14.78 ± 7.39 (15.5)	16.07 ± 4.41 (13.8)
12-24 hr	11.30 ± 2.45 (14.7)	12.02 ± 7.00 (12.6)	16.58 ± 5.40 (14.2)
Total	48.19 (62.7)	64.66 (67.8)	77.24 (66.2)
Cage wash	1.18 ± 0.16 (1.5)	1.52 ± 0.80 (1.6)	1.72 ± 0.80 (1.5)
Feces 0-6 hr	0.07 ± 0.03	0.20 ± 0.09	0.59 ± 0.81
6-12 hr	0.91 ± 0.07	0.82 ± 0.85	1.18 ± 0.51
12-24 hr	2.00 ± 0.46	3.42 ± 3.26	2.64 ± 1.80
Total	2.97 (3.9)	4.44 (4.7)	4.41 (3.8)
Total Excreted [c]	52.34 (68.1)	70.62 (74.0)	83.37 (71.4)
Carcass wash [d]	0.14 (0.2)	0.16 (0.2)	0.42 (0.4)
Carcass + tissues	24.35 (31.7)	24.64 (25.8)	32.98 (28.2)
Total Absorbed [e]	76.69 (99.8)	95.26 (99.8)	116.35 (99.6)
Total Recovery	76.83 (100)	95.42 (100)	116.76 (100)

[a] Data obtained from pages 42-45 in the study report; data are the average (±SD) of 3-5 rats/subgroup.

[b] Values listed in parentheses are the %dose corrected for the overall recovery. These values were calculated by the reviewer using the following formula: (%dose in matrix ÷ Total recovery) × 100.

[c] The total excreted was calculated by the reviewer and includes the %dose in urine, cage wash and feces.

[d] Includes radioactivity from the soapy and DI water washes of the carcass and the dry sponge wipes.

[e] The total absorbed was calculated by the reviewer and includes the %dose in carcass, tissues and excreta.

NS = not sampled.

TABLE 5: Recovery of radioactivity in tissues and excreta of female rats following a single 6-hour inhalation exposure of [^{14}C] Copper Omadine at 1.5 mg/m^3 [a]

Sample/interval	Percent of administered radioactive dose [b]		
	1 Exposure	5 Exposures	10 Exposures
Expired air	NS	NS	NS
Urine 0-6 hr	35.46 ± 21.45 (25.1)	24.36 ± 5.75 (26.2)	21.88 ± 4.26 (31.8)
6-12 hr	26.51 ± 15.44 (18.8)	16.73 ± 7.62 (18.0)	13.37 ± 4.79 (19.5)
12-24 hr	23.21 ± 9.18 (16.5)	14.18 ± 3.53 (15.3)	8.94 ± 1.69 (13.0)
Total	85.18 (60.4)	55.27 (59.5)	44.19 (64.3)
Cage wash	2.69 ± 0.95 (1.9)	1.52 ± 0.75 (1.6)	1.43 ± 1.13 (2.1)
Feces 0-6 hr	0.2 ± 0.2	0.5 ± 0.4	0.1 ± 0.1
6-12 hr	0.6 ± 0.6	1.3 ± 1.9	0.75 ± 0.38
12-24 hr	3.5 ± 1.7	2.7 ± 0.6	2.03 ± 0.55
Total	4.34 (3.1)	4.44 (4.8)	2.91 (4.2)
Total Excreted [c]	92.21 (65.4)	61.23 (65.9)	48.62 (70.7)
Carcass wash [d]	1.5 (1.1)	1.0 (1.1)	0.5 (0.7)
Carcass + tissues	47.26 (33.5)	30.62 (33.0)	19.71 (28.7)
Total absorbed [e]	139.47 (98.9)	91.85 (98.9)	68.33 (99.4)
Total Recovery	141.01 (100)	92.85 (100)	68.73 (100)

[a] Data obtained from pages 42-45 in the study report; data are the average (±SD) of 4-5 rats/subgroup.

[b] Values listed in parentheses are the %dose corrected for the overall recovery. These values were calculated by the reviewer using the following formula: (%dose in matrix ÷ Total recovery) × 100.

[c] The total excreted was calculated by the reviewer and includes the %dose in urine, cage wash and feces.

[d] Includes radioactivity from the soapy and DI water washes of the carcass and the dry sponge wipes.

[e] The total absorbed was calculated by the reviewer and includes the %dose in carcass, tissues and excreta.

NS = not sampled.

B. METABOLITE CHARACTERIZATION STUDIES:

No analyses of ^{14}C-residues in urine and feces were conducted in this metabolism study. Agency guidelines for a Tier I rat metabolism study (OPPTS Guideline 870.7485) require the identification and/or characterization of ^{14}C-resdiues in excreta.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

Following a 6-hour inhalation exposure with [^{14}C] copper omadine at 0.53 and 1.49 mg/m^3 (106 and 99% target concentration), the maximum concentration of radioactivity in the plasma was detected at 0.5 to 1 hour post-exposure. For the low-dose group, the maximum plasma concentration averaged 0.052 µg/g and was similar regardless of the number of prior exposures. For the high-dose group, the maximum plasma concentrations ranged from 0.100 to 0.185 µg/g, with the maximum concentration decreasing with increasing numbers of exposures to the non-labeled test substance.

Radioactivity was detected in all tissues collected at 24 hours post-exposure, with the highest concentration in liver and the lowest concentration in brain regardless of the concentration of test substance in the exposure atmosphere or the total number of exposures. The plasma, tissue, and carcass concentrations appeared to increase with a decreasing number of exposures in the high-dose group. Radioactivity was primarily excreted in the urine (approximately 64% of the AD by 24 hours post-exposure) and to a lesser degree in the feces (<5% of the AD). The percent recovery in the excreta, tissues, and remaining carcass averaged 96% for the low-dose group and 100% for the high-dose group.

B. REVIEWER COMMENTS:

Following a single 6-hour exposure to [^{14}C] copper omadine at concentrations of 0.53 and 1.49 mg/m^3, maximum concentrations of radioactivity in the plasma were observed at 0.5 or 1 hour post-exposure. Maximum plasma concentrations were similar for the low-dose subgroups (0.043-0.060µg/g) regardless of the number of prior exposures. However, for the high-dose group, the maximum plasma concentrations decreased from 0.185 to 0.100 µg/g as the number of prior exposures to the non-labeled test substance increased.

Although the average recovery of the calculated dose from the two groups was acceptable (96 and 100%), there was a high degree of variability in the recoveries among animals and subgroups, making comparison between the subgroups problematic. Average recoveries from the subgroups ranged from 68.7 to 141%. However, rather than being attributable to the poor recovery of the administered dose, this variability is most likely due to differences in the respiration rates between animals, which resulted in differences in the ^{14}C-doses achieved for each animal. Therefore, for comparison of the subgroups, the %AD in the various matrices were normalized to a recovery of 100% for each subgroup.

The radioactive dose was almost completely absorbed regardless of the number of pre-treatment days. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥98.9% of the AD for both dose groups. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for ≤1.1% of the AD. By 24 hours post-exposure, the pattern of excretion was similar for both dose groups. Regardless of the number of previous exposures, urinary excretion accounted for 62.7-67.8% of the AD for the low-dose group and 59.5-64.3% of the AD for the high-dose group, with the greatest amount being excreted in the urine within 12 hours of exposure. Fecal elimination was a minor route of excretion, accounting for ≤4.8% of the AD in all subgroups. Radioactivity remaining in the carcass and tissues at 24 hours post-exposure accounted for 25.8-33.5% of the AD. Although exhaled air was not collected for radioassay, the overall recoveries indicate that exhaled air is unlikely to be a substantial route of excretion.

For both dose groups, the relative distribution of the radioactive dose among tissues was similar. At 24 hours post-exposure, concentrations of ^{14}C-residues were highest in liver and kidney and lowest in brain, spleen and lungs. For the low-dose group, the concentration of radioactivity in a given tissue was similar regardless of the number of previous exposures. However, for the high-dose group, concentrations of ^{14}C-residues in tissues decreased significantly ($p<0.05$) as the number of prior exposures increased. For both dose groups, the

liver (2.6-5.0% AD) and carcasses (20.2-28.9% AD) accounted for the majority of the dose remaining in the body at 24 hours post-exposure.

C. STUDY DEFICIENCIES:

The following deficiencies were noted in the rat metabolism study:

- Exhaled air was not collected for radioassay. However, the overall recoveries of radioactivity indicate that exhaled air is unlikely to be a substantial route of excretion.
- The recovery of the "calculated dose" was highly variable between the individual rats and subgroups, and no explanation was provided to account for this variability.
- No analysis on conducted on ^{14}C-residues in the excreta as is normally recommended in a Tier I metabolism study.

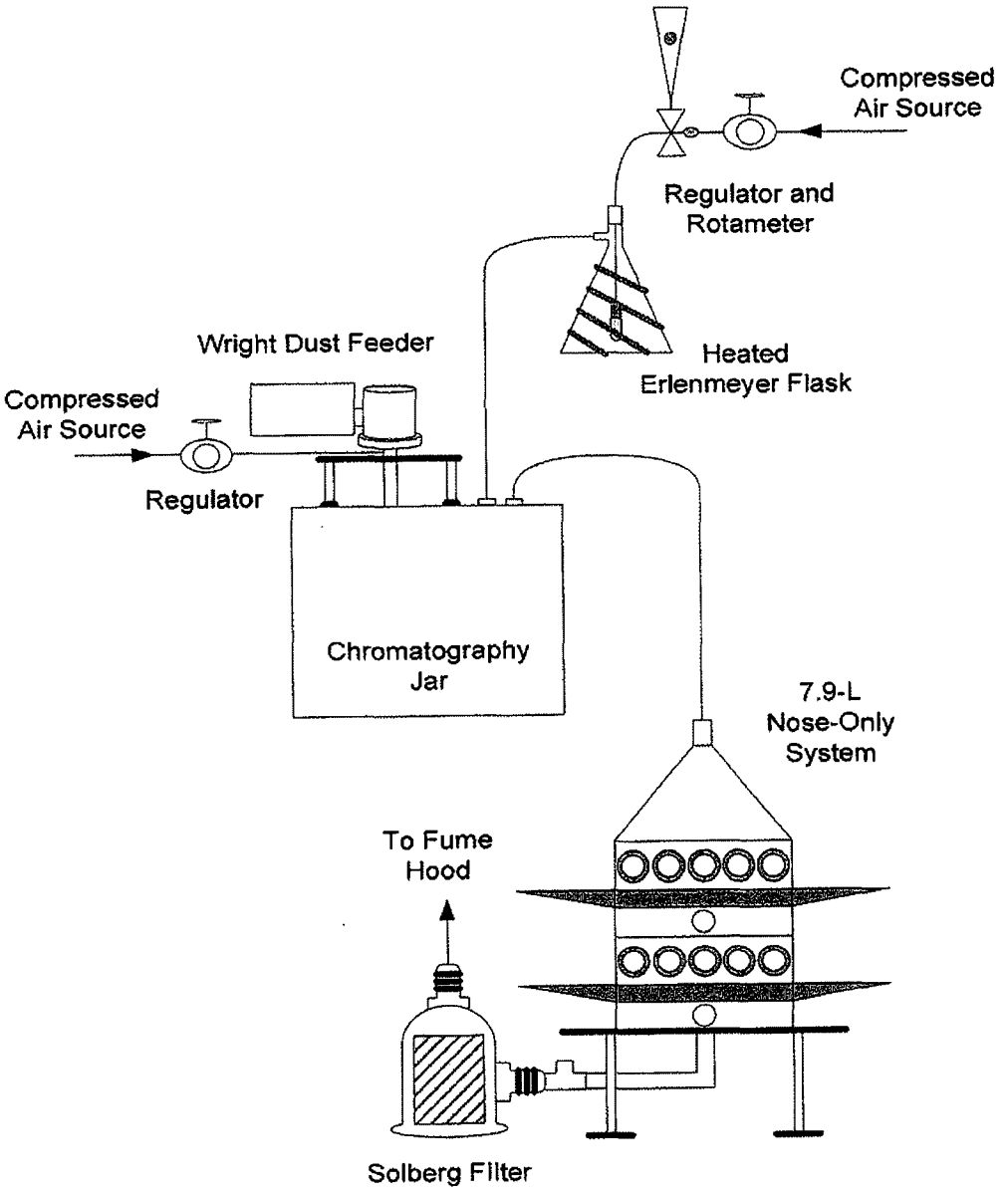
This metabolism study in the rat is classified **acceptable/non-guideline.** The study was conducted according to a protocol submitted by the registrant and reviewed by the Agency for the purpose of examining absorption, disposition, and excretion of copper pyrithione by the inhalation route and to compare these results to a similar study conducted with zinc pyrithione (MRID 48006402) for bridging purposes. As ~ 62.7-67.8% of the recovered dose was excreted in urine, it would be useful to characterize the nature of the residue in urine for comparative purposes to the existing metabolism/kinetic data on oral exposure to zinc pyrithione.

Sign-off Date : 02/15/11
DP Barcode Nos.: D375749 and D369393

TXR No. : 1,003,204

Appendix I. Inhalation Exposure System.

FIGURE 1: ATMOSPHERE GENERATION AND EXPOSURE SYSTEM



EPA Reviewer: Jonathan Chen, Ph.D.
RASSB, Antimicrobial Division (7510P)
Secondary Review: Tim McMahon, Ph.D.
Senior Scientist, Antimicrobial Division (7510P)

Signature: Jonathan Chen
Date: 02/15/2011
Signature:
Date: 2/15/11
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Metabolism – Rat (registrant submitted protocol).

PC CODE: 088002 **DP BARCODE:** 375749

TEST MATERIAL (PURITY): Zinc Omadine (98.1% a.i.)

SYNONYMS: Zinc pyrithione

CITATION: Thomas, J. A. (2010) Inhalation of ^{14}C-Zinc Omadine. WIL Research Laboratories, LLC, Ashland, OH. Report number: WIL-544011, February 19, 2010. MRID 48006402. Unpublished.

SPONSOR: Arch Chemicals, Inc., 350 Knotter Dr., Cheshire, CT, 06410 USA

EXECUTIVE SUMMARY:

In a metabolism study (MRID 48006402), [pyridinyl-2,6-^{14}C] zinc omadine (100% radiochemical purity, lot# 3620139) was administered to two groups of jugular vein-cannulated, female Sprague-Dawley (Crl:CD) rats (15 rats/group) by inhalation as an aerosolized dust at target concentrations of 0.5 and 1.5 mg/m^3. All animals received a single, 6-hour exposure to the ^{14}C-test substance using a dynamic, nose-only inhalation exposure system. Each dose group was divided into three subgroups (5 rats/subgroup), which received daily 6-hour exposures to non-labeled zinc omadine (98.1% ai., lot# 0108244691) at target concentrations of 0.5 and 1.5 mg/m^3 for either 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. The actual concentrations for the ^{14}C-test substance were 0.51 and 1.60 mg/m^3 for the low- and high-dose groups, respectively. Based on the 6-hour exposure, these concentrations were calculated to be equivalent to doses of 0.145 and 0.463 mg/kg body weight, assuming a respiration rate of 0.8 L/minute/kg body weight for the rats. The average concentrations of non-labeled test substance during the 4- and 9-day pretreatments were respectively 0.50 and 0.53 mg/m^3 for the low-dose group and 1.43 and 1.47 mg/m^3 for the high-dose group.

Following exposure to the [^{14}C] zinc omadine, blood samples were collected from each animal at 0.25, 0.5, 1.0 and 24 hours post-exposure, and urine, feces and cage wash samples were collected from 0-24 hours. Animals were sacrificed at 24 hours post-exposure, and the carcasses were washed and rinsed to assess external exposure. Selected tissues and the residual carcass were then collected for radioassay.

Maximum concentrations of radioactivity were observed in the plasma at 0.5 or 1 hour post-exposure. Maximum plasma concentrations were similar for the low-dose subgroups (0.027-0.035 μg/g) regardless of the number of prior exposures. However, for the high-dose group, the maximum plasma concentrations decreased from 0.233 to 0.110 μg/g as the number of exposures to non-labeled test substance increased.

The radioactive dose was almost completely absorbed regardless of the number of prior exposures. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥96.5% of the administered dose (AD) for both dose groups. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for ≤3.5% of the AD. Regardless of the number of previous exposures, urinary excretion accounted for 50.8-53.4% of the AD for the low-dose group and 46.7-52.0% of the AD for the high-dose group, with the greatest amount being excreted in the urine within 12 hours of exposure. Fecal elimination was a minor route of excretion, accounting for ≤4.8% of the AD in all subgroups. Radioactivity remaining in the carcass and tissues at 24 hours post-exposure accounted for 39.4-47.1% of the AD.

For both dose groups, radioactivity was detectable in all tissues at 24 hours post-exposure, and the relative distribution of the radioactive dose was similar among tissues. Concentrations of ^{14}C-residues were highest in the liver (low dose – 0.104-0.154 μg/g; high dose – 0.309-0.521 μg/g) and lowest in brain, spleen and lungs (low dose – 0.012-0.021 μg/g; high dose – 0.037-0.118 μg/g). For the low dose subgroups, the concentration of radioactivity in a given tissue was similar regardless of the number of previous exposures. However, for the high-dose group, concentrations of ^{14}C-residues in tissues decreased as the number of prior exposures increased. For both dose groups, the liver (2.9-4.4% AD) and carcasses (34.9-41.6% AD) accounted for the majority of the dose remaining in the body at 24 hours post-exposure.

This metabolism study in the rat is classified **acceptable/non-guideline.** The study was conducted according to a protocol submitted by the registrant and reviewed by the Agency for the purpose of examining absorption, disposition, and excretion of zinc pyrithione by the inhalation route and to compare these results to a similar study conducted with copper pyrithione for bridging purposes. As ~48-53% of the recovered dose was excreted in urine, it would be useful to characterize the nature of the residue in urine for comparative purposes to the existing metabolism/kinetic data on oral exposure to zinc pyrithione.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was conducted in compliance with EPA (40 CFR Part 160), and OCED [C(97) 186/Final)] Good Laboratory Practices and the standard operating procedures of WIL Research Laboratories, LLC. The only notable protocol deviation in the study involved the possible misidentification of the cage wash samples. However, because the results from the cage wash samples were similar among animals, this deviation did not have an adverse impact on the study findings.

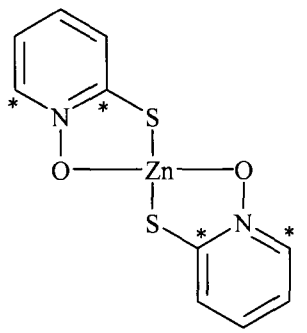
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	[Pyridinyl-2,6-^{14}C] Zinc omadine
Radiochemical purity:	99.6% (determined by HPLC)
Specific activity:	9.81 mCi/mmol (30.88 ΦCi/mg)
Lot/batch #:	3620139
Non-Radiolabeled test material:	Zinc omadine (zinc pyrithione)
Description:	(e.g. technical, white powder, stable at room temperature)
Lot/batch #:	0108244691
Purity:	98.1 ± 0.1 % a.i. (determined by iodometric titration)
Contaminants:	NA
CAS#:	13463-41-7
Molecular weight	317.72

Structure:



* indicates position of ^{14}C-label

2. Vehicle and/or positive control: not applicable.

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD[SD]); jugular vein-cannulated females
Age/weight at study *initiation*:	168-197 g/ 7 weeks
Source:	Group 1 – Charles River Laboratories (Portage, MI) Group 2 – Charles River Laboratories (Raleigh, NC)
Housing:	Individually in suspended wire mesh cages during non-exposure periods. Following exposure to the ^{14}C-labeled test substance, rats were housed individually in plastic metabolism cages suitable for the separate collection of urine and feces.
Diet:	Certified Rodent LabDiet® 5002; PMI Nutrition International, LLC; *ad libitum*
Water:	Reverse-osmosis treated water, *ad libitum*
Environmental conditions:	**Temperature:** 22 ± 3EC **Humidity:** 50 ± 20% **Air changes:** 10 /hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	≥5 days

4. Preparation of test substance used for exposure: The animals were exposed directly to the undiluted non-labeled and ^{14}C-labeled zinc omadine as an aerosolized dust.

B. STUDY DESIGN AND METHODS:

The objective of the study was to determine the absorption, distribution, and excretion profile of [^{14}C] zinc omadine® in female rats following a single nose-only inhalation exposure which was conducted after either 0, 4, or 9 prior nose-only inhalation exposures to non-labeled zinc omadine®. The metabolism of [^{14}C] zinc omadine was evaluated at target concentrations of 0.5 and 1.5 mg/m^3.

1. **Study dates:** Start: July 23, 2009; End: January 8, 2010

2. **Group arrangements:** Two groups of jugular vein-cannulated female rats (15 rats/group) were exposed to [^{14}C] zinc omadine (100% radiochemical purity) as a single, nose-only inhalation for 6 hours at target concentrations of 0.5 and 1.5 mg/m^3. Each group was subdivided into three subgroups (5 rats/subgroup), which received daily 6-hour exposures of non-labeled zinc omadine (98.1% ai.) for either 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. Animals were assigned randomly assigned to the test groups noted in Table 1.

   No rationale was provided as to why the 0.5 and 1.5 mg/m^3 concentrations were selected for the metabolism study. However, this study was conducted concurrently with a 4-week inhalation toxicity study of zinc omadine on rats (MRID 48006404) which utilized target exposure levels of 0.5, 1.5 and 5.0 mg/m^3.

TABLE 1: Dose groups for [^{14}C] Zinc Omadine Metabolism studies using Inhalation Exposure

Test group	Subgroup	Exposure concentration (mg/m^3)		Number of exposures [b]	Number females	Remarks
		Target	Actual [a]			
Group 1	A	0.5	0.51 ± 0.10	10	5	Blood was sampled from each rat at 0.25, 0.5, 1 and 24 hours post-^{14}C-dose. Urine and feces were collected from 0-24 hours post-exposure. Animals were sacrificed at 24 hours post-exposure, and selected tissues were collected for radioassay.
	B	0.5	0.51 ± 0.10	5	5	
	C	0.5	0.51 ± 0.10	1	5	
Group 2	A	1.5	1.60 ± 0..38	10	4 [c]	
	B	1.5	1.60 ± 0..38	5	5	
	C	1.5	1.60 ± 0..38	1	5	

[a] Data obtained from page 36 in the study report.

[b] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled zinc omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to exposure to the ^{14}C-test substance. Note that the schedule for exposure was 5 days/week; therefore, the rats receiving 10 doses were treated over a 2-week period.

[c] One animal from subgroup 2A died following 5 days of exposure to non-labeled zinc omadine.

3. **Dosing:** The non-labeled test substance was administered as a dust aerosol via nose-only inhalation for 6 hours per day for either 0, 4 or 9 days, followed by a single exposure with the ^{14}C-zinc omadine as an aerosolized dust via nose-only inhalation for 6 hours. The exposure system is described in the following section.

   Prior to the initial exposure, the animals were acclimated to restraint in nose-only exposure restraint tubes by increasing the restraint time over the 5 day acclimation period, from 1 hour on the first day to 6 hours by the fifth day. Following each restraint period, animals were observed for clinical signs of injury or stress. Animals were held in the restraint tubes for 25

to 60 minutes prior to the initiation of exposure. Animals were weighed prior to exposure to the non-labeled test substance and again prior to exposure to the ^{14}C-test substance.

For each exposure, the animals were placed into exposure restraint tubes, placed on the nose-only system, exposed for 6 hours, and then returned to their home cages. Animals were housed individually in plastic mesh cages during non-exposure hours. Food and water were withheld during the exposure period. Immediately following the 6-hour exposure to the [^{14}C] zinc omadine, the rats were transferred to plastic metabolism cages.

4. **Generation of the test atmosphere / chamber description:** A diagram of the test atmosphere generation system and exposure chamber used for exposure is included in Appendix I.

Exposures to the non-labeled zinc omadine were conducted using a 14.1-L conventional dynamic nose-only exposure system (designed and fabricated by WIL Research Laboratories, LLC) with synthetic rubber grommets in exposure ports to engage animal holding tubes. Exposure to the [^{14}C] zinc omadine were conducted using a similar 7.9-L nose-only exposure system.

Air supplied to the nose-only system was provided from a dry compressed air source. All test atmosphere exhaust passed through the facility exhaust system, which consisted of charcoal- and HEPA-filtration. Exposure chamber temperature, relative humidity, and chamber ventilation rate were continually monitored and manually recorded at approximately 60-minute intervals during the exposure. The mean temperature and relative humidity were set for 19-25°C and 30-70%, respectively. During the exposures, actual mean temperatures were 20-21°C and the relative humidity was 41-56%.

For generation of the dust aerosol atmosphere, the test substance was metered and aerosolized using a Wright Dust Feeder (model WDF-II, BGI, Inc., Waltham, MN) and speed controller (model F-352-BM, Electro-Craft Servo Products, Robbins & Myers, Inc., Hopkins, MN), which delivered the test substance aerosol at a constant rate into a 4.9-L chromatography jar. The WDF was equipped with a 1.3-cm^3 stainless steel cup, which was packed with the test substance. Dry compressed air was supplied to the WDF at a rate of 13.7 LPM to deliver test substance aerosol to the chromatography jar where large particles were removed prior to entering the nose-only exposure system. Humidified air was also added to the chromatography jar at a rate of 35 LPM using a regulator and flowmeter. The resulting aerosol from the chromatography jar was then delivered to the nose-only exposure system through ¾-inch ID anti-static tubing at a rate of ca. 48.7 LPM. The actual flow rates for the exposures were 48.7-56.8 LPM.

Particle size determination – Aerosol particle size determinations were conducted at least once for each sub-group using a 7-stage stainless steel cascade impactor. Pre-weighed, 22-mm stainless steel discs coated with a collection solution were used as the collection substrates for each stage and a 25-mm glass-fiber filter was used in the tail cup. During the non-labeled test substance exposures, samples were collected at approximately 1.8 LPM for 720 and 360 minutes for the 0.5 and 1.5 mg/m^3 exposures, respectively. For the labeled test substance exposures, samples were collected at approximately 2 LPM for 402 and 399

minutes for the 0.5 and 1.5 mg/m^3 exposures, respectively. The substrates were re-weighed and the particle size was calculated based on the impactor stage cut-offs, with the aerosol size being expressed in terms of the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). The particle size determinations for the exposures to non-labeled test substance are listed below:

Subgroup	Concentration (mg/m^3)	# exposures	# samples	Mean MMAD (µm)	Mean GSD (µm)
1A	0.5	9	3	1.3	1.89
1B	0.5	4	2	1.2	1.84
2A	1.5	9	2	1.6	2.01
2B	1.5	4	1	1.4	1.90

For the single 6-hour exposure to the ^{14}C-labeled test substance, which was conducted on the same day for the subgroups in a given dose group, the MMAD and GSD were respectively 1.3 and 1.91 µm for the 0.5 mg/m^3 dose group and 1.4 and 1.90 µm for the 1.5 mg/m^3 dose group.

Test atmosphere concentrations – Actual exposure concentrations were determined using standard gravimetric methods for exposure to both the non-labeled and ^{14}C-labeled test substances. Samples were collected on pre-weighed, 25-mm glass-fiber filters held in open-faced filter holders positioned in the animal breathing zones of the nose-only exposure systems. Following sample collection, the filter was re-weighed and the concentration calculated as the filter weight difference divided by the sample volume. For the exposures to the non-labeled test substance, 2 or 3 samples were collected for each daily exposure and the mean concentration was reported for each day. For the low-dose group, the average daily concentration (±SD) was 0.53 ± 0.062 mg/m^3 for the 9-day exposure subgroup and 0.50 ± 0.008 mg/m^3 for the 4-day exposure subgroup. For the high-dose group, the average daily concentration (±SD) was 1.47 ± 0.122 mg/m^3 for the 9-day exposure subgroup and 1.43 ± 0.150 mg/m^3 for the 4-day exposure subgroup.

On the day of exposure to the ^{14}C-labeled test substance, 7 filter samples were collected for each dose group. The average concentration (±SD) was 0.51 ± 0.101 mg/m^3 for the low-dose group and 1.60 ± 0.38 mg/m^3 for the high-dose group. Assuming a respiratory rate of 0.8 L/minute/kg body weight for the rats, the calculated daily dose for the 6-hour exposure was equivalent 0.147 mg/kg for the low-dose group and 0.461 mg/kg for the high-dose group.

Exposure concentrations for the ^{14}C-label test substance were also determined radiometrically. The filters were extracted by vortexing and sonication with methanol, and the resulting extracts were radioassayed in duplicate by liquid scintillation counting (LSC). Based on these radioassays, the average exposure concentration (±SD) was 0.51 ± 0.10 mg/m^3 for the low-dose group and 1.60 ± 0.37 mg/m^3 for the high-dose group. The results from the gravimetric analysis and radioassays were in close agreement.

5. **Clinical observations:** All animals were observed for mortality and morbidity twice daily throughout the study.

6. **Sample collection:** Following the 6-hour inhalation exposure to the [^{14}C] zinc omadine, blood samples were collected from each animal at 0.25, 0.5 and 1 hour post-exposure via the jugular vein cannula or from the retro-orbital sinus. For collections from the retro-orbital sinus, animals were anesthetized with inhaled isoflurane. Final blood samples were also collected after sacrifice (24-hour) from the vena cava. Blood samples were collected into tubes containing an anticoagulant and were centrifuged to obtain plasma samples for radioassay. Samples of urine and feces were collected over ice at the following intervals: 0-6, 6-12 and 12-24 hours post-exposure. At each collection interval, cage surfaces were rinsed with deionized water, and the cage wash samples were retained for separate analysis. All rats were euthanized at 24 hours post-exposure by the inhalation of carbon dioxide. Each carcass was washed twice with Exodontia sponges wetted with a 1:50 (v/v) solution of Ivory liquid soap:deionized water, then rinsed twice with Exodontia sponges wetted with deionized water, and finally wiped dry with two Exodontia sponges. After drying, the final blood sample was collected from each animal along with the following tissues: lung, liver, kidney, spleen, brain, stomach, and gastrointestinal (GI) tract. The remaining carcass was weighed and retained. All samples were held on wet ice until storage at -20°C.

7. **Radioassay:** Subsamples of plasma, urine, cage wash, and cage rinse were radioassayed in duplicate for total radioactivity directly by LSC without further processing. Tissue and carcass samples were chopped and homogenized and then radioassayed in duplicate by combustion/LSC. Fecal samples were homogenized with distilled water (2:1) and radioassayed in duplicate by combustion/LSC. Filter samples from the nose-only exposure system were extracted by vortexing and sonication with methanol. The resulting methanol extract was then radioassayed in duplicate by LSC. The wash sponges were digested in 50% sulfuric acid:methanol (50:4, v/v), diluted with water and then radioassayed directly by LSC.

   The recovery of radioactivity from the various sample types was validated by fortifying samples collected from control animals with known amounts of [^{14}C]zinc omadine. The recovery of radioactivity from duplicate, fortified samples of urine, feces, tissues, plasma, cage washes, and sponge digests ranged from 92.1 to 104%, with the exceptions of spleen (82.1% recovery), brain (86.1% recovery), and lungs (81.7% recovery). The calculated limits of quantitation (LOQ) for the radioassays ranged from 0.00007 µg/g for the cage washes to 0.0073 µg/g for plasma.

8. **Metabolite characterization studies:** Although samples of urine and feces were collected in this study, no analyses were performed to identify or characterize the nature of the ^{14}C-resdiues in excreta.

9. **Statistics:** Means with standard deviations (SD) were calculated for the various parameters measured for each dose subgroup (i.e. concentrations of radioactivity in tissues, % AD in tissues, carcass and excreta).

II. RESULTS:

A. PHARMACOKINETIC STUDIES:

1. **Clinical Observations:** A single female was found dead following 5 days of exposure to non-labeled zinc omadine at 1.5 mg/m^3. A complete necropsy was performed, and there were no remarkable external observations. Internal evaluations noted the presence of clear fluid in the thoracic cavity, the enlargement and discoloration (dark red) of the mediastinal lymph nodes, and discoloration (dark red) of the kidneys. The cause of death was undetermined and no findings could be attributed to the test substance. An additional female was also found dead prior to any exposures to the test substance. The author noted that similar deaths of three untreated animals were observed during the metabolism study with [^{14}C] copper omadine (MRID 48006401). The study author attributed the deaths to factors associated with the presence of an in-dwelling venous cannula, and the stress from confinement in the nose-only exposure.

1. **Absorption:** For both the low- and high-dose groups, the radioactive dose was almost completely absorbed regardless of the number of pre-treatment days. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥98.9% of the AD for the low-dose group and ≥96.5% of the AD for the high-dose group (See Tables 4 and 5). Even if radioactivity associated with the lungs is not considered to be absorbed, the levels of absorption would still be >96% of the AD, as the lungs accounted for ≤0.1% of the AD. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for <1.25% of the AD for the low-dose group and <3.0% of the AD for the high-dose group.

2. **Plasma kinetics:** The maximum observed concentration of radioactivity in plasma occurred at 0.5 or 1 hour post-exposure regardless of the test substance exposure concentration or the number of previous exposures (Table 2). The maximum concentrations of radioactivity were 0.027-0.035 µg/g for the low-dose group and 0.110-0.233 µg/g for the high-dose group. By 24 hours post-exposure, plasma concentrations decreased to 0.016-0.021 µg/g for the low-dose group and 0.046-0.090 µg/g for the high-dose group.

   The increase in plasma concentrations for the high-dose group generally reflected the 3-fold increase in the dose level. Compared to the low-dose group, the concentrations of plasma radioactivity for the high-dose subgroups were generally 7.8-fold higher after 1 exposure, 3.6-fold higher after 5 exposures, and 3.9-fold higher after 10 exposures.

   There was no apparent correlation between plasma concentration and the number of previous exposures for the 0.5 mg/m^3 exposure group. However, for 1.5 mg/m^3 dose group, plasma concentrations of radioactivity decreased at each sampling interval with an increasing number of exposures.

TABLE 2: Distribution of radioactivity in tissues/organs of female rats 24 hours following a single 6-hour inhalation exposure of [^{14}C] Zinc Omadine at 0.5 or 1.5 mg/m^3 [a]

Sampling time (hr)	Mean concentration of radioactivity in plasma (µg equivalents/g)					
	0.5 mg/m^3			1.5 mg/m^3		
	1 exposure	5 exposures	10 exposures	1 exposure	5 exposures	10 exposures
0.25	0.020 ± 0.005	0.025 ± 0.015	0.020 ± 0.004	0.182 ± 0.091	0.110 ± 0.034	0.104 ± 0.040
0.5	0.027 ± 0.008	0.033 ± 0.012	0.026 ± 0.010	0.233 ± 0.149	0.121 ± 0.030	0.110 ± 0.050
1.0	0.026 ± 0.006	0.035 ± 0.013	0.029 ± 0.012	0.204 ± 0.130	0.124 ± 0.036	0.095 ± 0.043
24	0.016 ± 0.004	0.021 ± 0.004	0.016 ± 0.005	0.090 ± 0.050	0.057 ± 0.009	0.046 ± 0.014
C_{max} (µg/g)	0.027	0.035	0.029	0.233	0.124	0.110
T_{max} (hr)	0.5	1	1	0.5	1	1

[a] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled zinc omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to ^{14}C-exposure. Data are the average of 4-5 rats/subgroup and were obtained from page 38 in the study report.

3. **Tissue distribution:** Concentrations of radioactivity in tissues at 24 hours post-exposure are summarized in Table 3. Following the 6-hour exposure to [^{14}C] zinc omadine at 0.5 mg/m^3, the mean concentrations of radioactivity at 24 hours post-exposure were highest in the liver (0.104-0.154 µg/g) for all subgroups and lowest in the brain, spleen and lungs (0.012-0.021 µg/g). The concentration of radioactivity in the remaining tissues and carcasses were similar, ranging from 0.022 to 0.072 µg/g. The concentration of radioactivity in any given tissue was similar regardless of the number of previous exposures. The liver accounted for 2.9-4.4% of the AD, the residual carcasses accounted for 34.9-35.8% of the AD, and the remaining tissues each accounted for ≤1.1% of the AD.

   Following the 6-hour exposure to [^{14}C] zinc omadine at 1.5 mg/m^3, the relative distribution of radioactivity among tissues was similar to the low-dose group; however, the high-dose group showed decreases in the tissue concentrations with an increasing number of prior exposures. For all high-dose subgroups, the mean concentrations of radioactivity at 24 hours post-exposure were highest in liver (0.309-0.521 µg/g) and lowest in the brain, spleen and lungs (0.037-0.118 µg/g). The concentration of radioactivity in the remaining tissues and carcasses were similar within each subgroup, ranging from 0.188-0.299 µg/g for 1 exposure, 0.117-0.248 µg/g for 5 exposures, and 0.062-0.195 µg/g for 10 exposures. Concentrations of radioactivity in all tissues decreased with an increased number of exposures. Compared to a single exposure, the concentrations in tissues and the carcass generally declined by 0.7x with 5 exposures, and by 0.5x with 10 exposures. As in the low-dose group, the liver (3.0-3.6% AD) and carcass (35.8-41.6% AD) accounted for majority of the dose remaining in the body at 24 hours post-exposure.

TABLE 3: Distribution of radioactivity in tissues/organs of female rats 24 hours following a single 6-hour inhalation exposure of [^{14}C] Zinc Omadine at 0.5 or 1.5 mg/m^3 [a]

Tissue/organ [b]	Concentration of radioactivity (µg equivalents/g)					
	0.5 mg/m^3			1.5 mg/m^3		
	1 exposure	5 exposures	10 exposures	1 exposure	5 exposures	10 exposures
Brain	0.012 ± 0.004	0.016 ± 0.003	0.015 ± 0.006	0.073 ± 0.039	0.044 ± 0.008	0.037 ± 0.011
Lung	0.016 ± 0.005	0.021 ± 0.005	0.019 ± 0.005	0.118 ± 0.086	0.057 ± 0.011	0.049 ± 0.013
Stomach	0.027 ± 0.011	0.034 ± 0.011	0.022 ± 0.006	0.188 ± 0.121	0.117 ± 0.059	0.062 ± 0.018
Kidney	0.048 ± 0.024	0.061 ± 0.011	0.047 ± 0.015	0.221 ± 0.142	0.161 ± 0.046	0.125 ± 0.029
Liver	0.135 ± 0.117	0.154 ± 0.030	0.104 ± 0.023	0.521 ± 0.309	0.427 ± 0.216	0.309 ± 0.104
Spleen	0.013 ± 0.003	0.017 ± 0.004	0.014 ± 0.005	0.076 ± 0.042	0.049 ± 0.009	0.040 ± 0.012
GI tract	0.038 ± 0.019	0.038 ± 0.014	0.036 ± 0.011	0.207 ± 0.127	0.143 ± 0.052	0.077 ± 0.025
Carcass	0.053 ± 0.019	0.072 ± 0.016	0.061 ± 0.030	0.299 ± 0.165	0.248 ± 0.155	0.195 ± 0.109

[a] Subgroups of rats (5 per subgroup) were pretreated with 6-hr daily inhalation exposures of non-labeled zinc omadine at 0.5 or 1.5 mg/m^3 for 0, 4 or 9 days prior to ^{14}C-exposure. Data are the average of 4-5 rats/subgroup and were obtained from pages 39 and 41 in the study report.

[b] All tissue and organ samples were collected at 24 hours post-^{14}C-exposure.

4. **Excretion:** The recovery and excretion of radioactivity following a 6-hour inhalation exposure to [^{14}C] zinc omadine at target concentrations of 0.5 and 1.5 mg/m^3 are presented in Tables 4 and 5, respectively. Although the overall recovery of the low- and high-dose groups averaged 93.4 and 106% of the AD, respectively, the overall recovery of the calculated dose varied considerably between the subgroups within each dose group. The recoveries ranged from 78.9 to 110.6% of the AD for the low-dose subgroups and from 85.2 to 141.8% of the AD for high-dose subgroups. The study author did not provide an explanation for the observed variability. However, the high degree of variability between animals is likely due to the differential respiration rates for the individual rats, resulting in a higher degree of variability in the achieved doses. The calculated dose for each animal assumed a standard respiration rate of 0.8 L/minute/kg body weight. In order to compare values across the subgroups, the amount of radioactivity (%AD) associated with each fraction was normalized such that the overall recovery for each subgroup was 100%.

   When normalized to account for the overall recovery in each subgroup, the pattern of excretion was similar for both dose groups. Regardless of the number of previous exposures, urinary excretion accounted for 50.8-53.4% of the AD for the low-dose group and 46.7-52.0% of the AD for the high-dose group by 24 hours post-exposure, and for both dose groups, the 0-6 hour urine fraction accounted for the largest fraction of radioactivity in the urine (low-dose – 22.2-26.9% AD; high-dose – 21.2-28.4% AD). By 24 hours post-exposure, fecal excretion accounted for ≤4.8% of the AD for all subgroups, and radioactivity remaining in the carcass and tissues accounted for 39.4-42.1% of the AD for the low-dose group and 40.5-47.1% of the AD for the high-dose group.

TABLE 4: Recovery of radioactivity in tissues and excreta of female rats following a single 6-hour inhalation exposure of [^{14}C] Zinc Omadine at 0.5 mg/m^3 [a]

Sample/interval	Percent of administered radioactive dose [b]		
	1 Exposure	5 Exposures	10 Exposures
Expired air	NS	NS	NS
Urine 0-6 hr	17.55 ± 3.10	26.58 ± 9.26	25.17 ± 9.91
6-12 hr	10.61 ± 6.04	14.18 ± 2.35	11.94 ± 3.55
12-24 hr	12.76 ± 4.40	15.45 ± 4.19	12.79 ± 3.54
Total	40.92 (51.9)	56.21 (50.8)	49.90 (53.4)
Cage wash	1.65 ± 0.47 (2.1)	2.23 ± 0.66 (2.0)	2.71 ± 1.34 (2.9)
Feces 0-6 hr	0.05 ± 0.02	0.10 ± 0.11	0.25 ± 0.42
6-12 hr	0.44 ± 0.26	1.75 ± 0.93	0.69 ± 0.88
12-24 hr	1.68 ± 1.05	3.43 ± 0.83	2.41 ± 0.86
Total	2.17 (2.7)	5.28 (4.8)	3.35 (3.6)
Total Excreted [c]	44.74 (56.7)	63.72 (57.6)	55.96 (59.9)
Carcass wash [d]	0.89 (1.1)	1.25 (1.1)	0.71 (0.8)
Carcass + tissues	33.26 (42.1)	45.61 (41.3)	36.83 (39.4)
Total Absorbed [e]	78.02 (98.9)	109.31 (98.9)	92.79 (99.2)
Total Recovery	78.91	110.56	93.50

[a] Data obtained from pages 43-46 in the study report; data are the average (±SD) of 5 rats/subgroup.

[b] Values listed in parentheses are the %dose corrected for the overall recovery. These values were calculated by the reviewer using the following formula: (%dose in matrix ÷ Total recovery) × 100.

[c] The total excreted was calculated by the reviewer and includes the %dose in urine, cage wash and feces.

[d] Includes radioactivity from the soapy and DI water washes of the carcass and the dry sponge wipes.

[e] The total absorbed was calculated by the reviewer and includes the %dose in carcass, tissues and excreta.

NS = not sampled.

TABLE 5: Recovery of radioactivity in tissues and excreta of female rats following a single 6-hour inhalation exposure of [^{14}C] Zinc Omadine at 1.5 mg/m^3 [a]			
Sample/interval	**Percent of administered radioactive dose [b]**		
	1 Exposure	**5 Exposures**	**10 Exposures**
Expired air	NS	NS	NS
Urine 0-6 hr	32.47 ± 16.93	20.84 ± 8.09	24.22 ± 7.36
6-12 hr	19.73 ± 13.60	12.00 ± 1.50	8.50 ± 1.71
12-24 hr	21.45 ± 12.52	13.13 ± 2.92	9.63 ± 4.69
Total	73.65 (52.0)	45.97 (46.7)	42.35 (49.7)
Cage wash	2.47 ± 1.37 (1.7)	1.58 ± 0.89 (1.6)	1.27 ± 0.53 (1.5)
Feces 0-6 hr	3.0 ± 6.2	0.3 ± 0.3	0.3 ± 0.4
6-12 hr	0.8 ± 0.9	0.3 ± 0.1	1.14 ± 1.03
12-24 hr	1.7 ± 1.4	1.4 ± 1.0	1.65 ± 0.88
Total	5.5 (3.9)	2.0 (2.0)	3.09 (3.6)
Total Excreted [c]	81.62 (57.6)	49.55 (50.3)	46.71 (54.8)
Carcass wash [d]	2.85 (2.0)	2.56 (2.6)	2.99 (3.5)
Carcass + tissues	57.36 (40.5)	46.36 (47.1)	35.46 (41.6)
Total absorbed [e]	138.90 (98.0)	95.94 (97.4)	82.18 (96.5)
Total Recovery	141.75	98.50	85.17

[a] Data obtained from pages 43-46 in the study report; data are the average (±SD) of 4-5 rats/subgroup.

[b] Values listed in parentheses are the %dose corrected for the overall recovery. These values were calculated by the reviewer using the following formula: (%dose in matrix ÷ Total recovery) × 100.

[c] The total excreted was calculated by the reviewer and includes the %dose in urine, cage wash and feces.

[d] Includes radioactivity from the soapy and DI water washes of the carcass and the dry sponge wipes.

[e] The total absorbed was calculated by the reviewer and includes the %dose in carcass, tissues and excreta.

NS = not sampled.

B. METABOLITE CHARACTERIZATION STUDIES:

No analyses of ^{14}C-residues in urine and feces were conducted in this metabolism study. Agency guidelines for a Tier I rat metabolism study (OPPTS Guideline 870.7485) require the identification and/or characterization of ^{14}C-resdiues in excreta.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

Following a 6-hour inhalation exposure with [^{14}C] zinc omadine at 0.51 and 1.60 mg/m^3 (103% and 107% target concentration, respectively), the maximum concentration of radioactivity in the plasma was detected at 0.5 to 1 hour post-exposure. For the low-dose group, the highest plasma concentration was 0.035 µg/g for the low-dose group and 0.233 µg/g for the high-dose group.

Radioactivity was detected in all tissues collected at 24 hours post-exposure, with the highest concentration in liver and the lowest concentration in brain regardless of the concentration of

test substance in the exposure atmosphere or the total number of exposures. The study auther concluded that there appeared to be no trend in the concentration of ^{14}C-resdiuses in plasma, tissue, or carcass related to the number of exposures to the unlabeled test substance at either exposure atmosphere concentration.

Radioactivity was primarily excreted in the urine (approximately 53% of the AD [not corrected] by 24 hours post-exposure) and to a lesser degree in the feces (<6% of the AD [not corrected]). The percent recovery in the excreta, tissues, and remaining carcass averaged 93% for the low-dose group and 106% for the high-dose group.

B. REVIEWER COMMENTS:

Following a single 6-hour exposure to [^{14}C] zinc omadine at concentrations of 0.51 and 1.60 mg/m^3, maximum concentrations of radioactivity in the plasma were observed at 0.5 or 1 hour post-exposure. Maximum plasma concentrations were similar for the low-dose subgroups (0.027-0.035 µg/g) regardless of the number of prior exposures. However, for the high-dose group, the maximum plasma concentrations decreased from 0.233 to 0.110 µg/g as the number of prior exposures to the non-labeled test substance increased.

Although the average recovery of the calculated dose from the two groups was acceptable (93 and 106%), there was a high degree of variability in the recoveries among animals and subgroups, making comparison between the subgroups problematic. Average recoveries from the subgroups ranged from 78.9 to 141.8%. However, rather than being attributable to the poor recovery of the administered dose, this variability is most likely the result of differences in the respiration rates between animals, which resulted in differences in the ^{14}C-doses achieved for each animal. Therefore, for comparison of the subgroups, the %AD in the various matrices were normalized to a recovery of 100% for each subgroup.

The radioactive dose was almost completely absorbed regardless of the number of pre-treatment days. When normalized for the percent recovery, the total radioactivity recovered in the urine, feces, carcass and tissues accounted for ≥96.5% of the AD for both dose groups. Radioactivity recovered from the exterior of the rat carcasses (carcasses washes and rinses) accounted for ≤3.5% of the AD. By 24 hours post-exposure, the pattern of excretion was similar for both dose groups. Regardless of the number of previous exposures, urinary excretion accounted for 50.8-53.4% of the AD for the low-dose group and 46.7-52.0% of the AD for the high-dose group, with the greatest amount being excreted in the urine within 12 hours of exposure. Fecal elimination was a minor route of excretion, accounting for ≤4.8% of the AD in all subgroups. Radioactivity remaining in the carcass and tissues at 24 hours post-exposure accounted for 39.4-47.1% of the AD. Although exhaled air was not collected for radioassay, the overall recoveries indicate that exhaled air is unlikely to be a substantial route of excretion.

For both dose groups, the relative distribution of the radioactive dose among tissues was similar. At 24 hours post-exposure, concentrations of ^{14}C-residues were highest in the liver and lowest in brain, spleen and lungs. For the low-dose group, the concentration of radioactivity in a given tissue was similar regardless of the number of previous exposures. However, for the high-dose group, concentrations of ^{14}C-residues in tissues decreased as the

number of prior exposures increased. For both dose groups, the liver (2.9-4.4% AD) and carcasses (34.9-41.6% AD) accounted for the majority of the administered dose remaining in the body at 24 hours post-exposure.

C. STUDY DEFICIENCIES:

The following deficiencies were noted in the rat metabolism study:

- Exhaled air was not collected for radioassay. However, the overall recoveries of radioactivity indicate that exhaled air is unlikely to be a substantial route of excretion.

- The short duration of the post-exposure period (24 hours) resulted in a substantial portion (39-47% AD) of the radioactivity remaining in the carcass and tissues. These residues were not subjected to further analysis.

- The recovery of the "calculated dose" was highly variable between the individual rats and subgroups, and no explanation was provided to account for this variability.

- No analysis was conducted on the ^{14}C-residues in excreta as is required for a Tier I metabolism study.

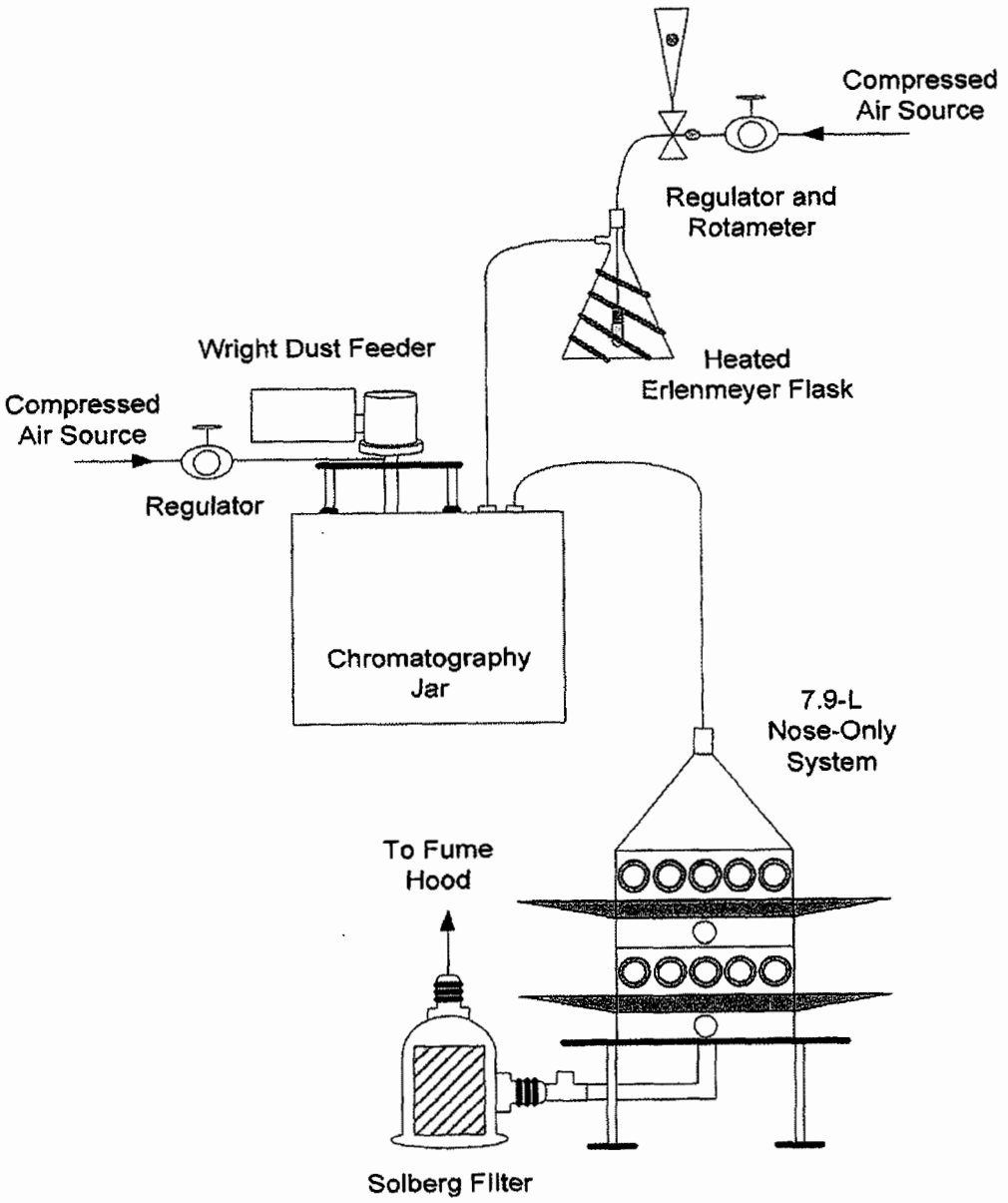
This metabolism study in the rat is classified **acceptable/non-guideline.** The study was conducted according to a protocol submitted by the registrant and reviewed by the Agency for the purpose of examining absorption, disposition, and excretion of zinc pyrithione by the inhalation route and to compare these results to a similar study conducted with copper pyrithione for bridging purposes. As ~48-53% of the recovered dose was excreted in urine, it would be useful to characterize the nature of the residue in urine for comparative purposes to the existing metabolism/kinetic data on oral exposure to zinc pyrithione.

Sign-off Date : 02/15/11
DP Barcode Nos.: D375749 and D369393

TXR No. : 1,003,204

Appendix I. Inhalation Exposure System.

FIGURE 1: ATMOSPHERE GENERATION AND EXPOSURE SYSTEM



EPA Reviewer: Jonathan Chen, Ph.D.
RASSB, Antimicrobials Division
Secondary Review: Tim McMahon, Ph.D.
Senior Scientist, Antimicrobials Division

Signature: Jonathan Chen
Date: 02/11/2011
Signature:
Date: 2/15/11
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity (registrant submitted protocol)

PC CODE: 088001 **DP BARCODE:** D375749

TEST MATERIAL (PURITY): Copper Omadine® (97% a.i.)

SYNONYMS: Copper-2-pyridinethio-1-oxide

CITATION: Kirkpatrick, Daniel T. (2009) A 4-Week inhalation toxicity study with emphasis on pulmonary effects of copper omadine in Sprague-Dawley rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544009, December 14, 2009. MRID 48006403. Unpublished.

SPONSOR: Arch Chemicals, 350 Knotter Drive, Cheshire, CT

EXECUTIVE SUMMARY: In a subchronic inhalation toxicity study (MRID 48006403), Copper Omadine® (97% a.i., Lot no. 0103239911) was administered as a dust aerosol to 15 Crl:CD(SD) rats/sex/concentration by nose-only exposure at concentrations of 0 (air), 0.5, 1.5, or 5.0 mg/m^3 (equivalent to analytical concentrations of 0, 0.0005, 0.0015, and 0.0049 mg/L) for 6 hours per day, 5 days/week for up to 4 weeks (up to 20 exposure days). Five rats/sex/concentration were euthanized following 1, 2, and 4 weeks of exposure and subjected to a gross necropsy. Selected organs were weighed and examined microscopically. A special emphasis was placed on the evaluation of pulmonary effects, including assessment of bronchoalveolar lavage fluid (BALF) parameters and microscopic examination of the lungs.

Test substance-related mortality was observed in the 0.005 mg/L group females. Two females (nos. 6047 and 6062) were found dead on Day 12, and one female (no. 6029) was found dead on Day 19. Test substance-related clinical observations noted for the animals found dead included hypoactivity, thin body condition, and body cool to touch. Additionally, female no. 6029 was noted with decreased defecation on Day 17, as well as dermal atonia and impaired muscle coordination on Day 18. The majority of these clinical observations were noted within 24 hours of death. However, the cause of death for these three females was undetermined. Microscopic findings in the lungs (broncho-interstitial pneumonitis characterized by subacute inflammation and an increase in alveolar macrophages) and skeletal muscle (atrophy and degeneration) were similar in nature and severity to that which occurred in females at the scheduled necropsy on Day 26. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

Test substance-related clinical observations were noted for the 0.005 mg/L group females surviving to scheduled termination. These findings included the following: thin body condition noted by Day 11 and continuing throughout the duration of the study, dermal atonia noted from Days 12 to 19, and pale extremities noted in a single animal on Day 20. Impaired use of right and left hindlimbs was noted for two animals ranging from Day 20 to 24. Other treatment-related clinical findings in this group were limited to yellow and/or red material on various parts of the body (including ocular, nasal, urogenital, and anal).

Treatment-related effects on body weights were observed in both sexes at 0.005 mg/L. In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased on Day 4 and continued to be decreased (not significant) throughout the remainder of the study. A significant ($p \leq 0.01$) body weight loss was noted for Days 0-4 in the 0.005 mg/L males (-10 g) compared to a gain of 11 g in the controls, and cumulative body weight gains were 42-56% lower than controls for all other intervals throughout the study; these decreases were statistically significant for all intervals, except for Days 0-25. In the 0.005 mg/L females, body weights were significantly ($p \leq 0.01$) decreased throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 90% lower ($p \leq 0.01$) than controls for Days 0-4, and cumulative body weight losses of 22-48 g were noted for each of the remaining intervals throughout the study in this group compared to body weight gains of 23-48 g in the control group.

Food consumption was decreased ($p \leq 0.05$) in the 0.0015 mg/L females for Days 0-4. Additionally at 0.005 mg/L, food consumption was decreased ($p \leq 0.01$) for Days 0-4 in the males and for Days 0-4, 4-11, and 11-18 in the females.

Treatment-related effects on BALF samples were found at 0.0015 and 0.005 mg/L in both sexes. Lactate dehydrogenase was increased over controls at 0.0015 mg/L in the males on Days 12 and 26 and in the females on Day 5 and at 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total protein levels were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total cell counts and the number of lymphocytes were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Day 5. Alveolar macrophages were increased in the 0.0015 and 0.005 mg/L males throughout the study and in the 0.005 mg/L females on Day 5. With the exception of the 0.005 mg/L females on Day 26, the number of neutrophils was increased throughout the study in the 0.0015 and 0.005 mg/L males and females.

Examination of the BALF leukocyte differential data showed that the proportion of neutrophils increased with concentration and time in both sexes at 0.0015 and 0.005 mg/L, with the exception of the 0.005 mg/L females on Day 26. The remaining three females exposed to 0.005 mg/L had apparently recovered (following the final exposure on Day 18), and neutrophils were not present in the BALF on Day 26. The proportion of lymphocytes was higher than controls in the 0.005 mg/L males and females on Days 5 and 12. Due to these increases in neutrophils and lymphocytes, the proportion of alveolar macrophages was lower in the 0.005 mg/L males on Days 5, 12, and 26 and females on Days 5 and 12. The proportion of alveolar macrophages was slightly lower than controls at 0.0015 mg/L in both sexes at all three intervals.
At 0.0015 mg/L, relative (to body weight) lung weights were increased ($p \leq 0.01$) over controls in the males on Day 12. The following increases ($p \leq 0.05$) in lung weights were observed in the

0.005 mg/L animals: (i) relative to body weight and relative to brain weight in the males and females at Day 5; (ii) absolute and relative to body weight in the males at Days 12 and 26; and (iii) relative to body weight in the females at Days 12 and 26. Terminal body weights were decreased ($p \leq 0.05$) at this concentration in the males on Day 5 and in the females on Days 12 and 26. Subacute inflammation was observed in the lungs in males at 0.0015 mg/L (4/15) and 0.005 mg/L (13/15) compared to controls (0/15) and in the females at 0.005 mg/L (9/12) compared to controls (1/15). The severity of subacute inflammation of the lungs ranged from minimal to moderate in the males and from minimal to mild in the females. Minimal alveolar macrophages were observed in the 0.0015 mg/L males (5/15) and females (3/15), and mild alveolar macrophages were found at 0.005 mg/L in the males (12/15) and females (9/12) compared to controls (0/15 males; 1/15 females). Perivasculitis was found in the 0.005 mg/L males (1/15) and females (2/12) compared to controls (0/15 males; 1/15 females). These findings were considered to be due to direct effects of the test material on the respiratory tract. With the exception of the perivasculitis in the females, the findings increased in severity with increasing concentration.

Enlarged bronchial lymph nodes were noted in one 0.005 mg/L female on Day 5, one 0.0015 mg/L male on Day 12, two 0.005 mg/L males on Day 12, one 0.005 mg/L female on Day 12, and one 0.005 mg/L male on Day 26. Enlarged mediastinal lymph nodes were noted in one 0.0015 mg/L male on Day 12, one 0.005 mg/L male on Day 12, two 0.005 mg/L females on Day 12, and one 0.005 mg/L male on Day 26. These findings, noted at gross necropsy, corresponded to lymphoid hyperplasia confirmed upon microscopic examination.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to moderate degeneration (4/15 treated vs 0/15 controls) and mild to moderate atrophy (5/15 treated vs 0/15 controls).

The LOAEL is 0.0015 mg/L based on decreased food consumption in the females, effects on BALF parameters (increased LDH, total protein, total cell counts, lymphocytes, alveolar macrophages, and neutrophils) and histopathology in the lungs (subacute inflammation and alveolar macrophages) in both sexes. The NOAEL is 0.0005 mg/L.

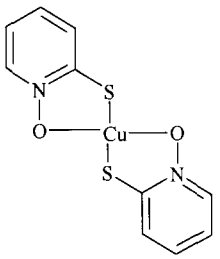
This 28-day study is classified as **acceptable/non-guideline.** This study was conducted after review of a protocol submitted by the registrant as part of discussions between the Antimicrobials Division and the registrant to determine toxic similarity between zinc pyrithione and copper pyrithione. The purpose of this study with copper pyrithione was to examine toxic effects of copper pyrithione by inhalation after 4 weeks, which included examination of lung bronchioalveolar lavage fluid after single or repeated inhalation exposures, and examination of lung histopathology. Certain parameters (microscopic examination of nasal passages, trachea, and larynx; neurobehavioral, ophthalmologic, and clinical pathology examinations) were not conducted in this study. A similar study has been conducted with zinc pyrithione (MRID 48006404).

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Copper Omadine®

Description:	Non-uniform olive-green powder
Lot #:	0103239911
Purity:	97% a.i.
Compound stability:	Samples of the test article were collected and analyzed prior to use on the study (103% pure) and after use on the study 48 days later (93.4% pure). Furthermore, concentration analyses were determined for each test atmosphere at least twice during each exposure period. The results of these analyses, shown in Table 1 of this DER, indicated that the animals were exposed to a consistent test concentration over the duration of the study.
CAS # of TGAI:	14915-37-8
Structure:	

2. Vehicle and/or positive control: Air

3. Test animals

Species:	Rat
Strain:	Crl:CD(SD)
Age/weight at study initiation:	Approximately 9 weeks old; 231-297 g males, 180-223 g females
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Individually in stainless steel wire mesh cages suspended above cage board
Diet:	Certified Rodent LabDiet 5002 (PMI Nutrition International, St. Louis, MO), *ad libitum*, except during exposure and overnight prior to termination
Water:	Reverse-osmosis filtered (on-site) water, *ad libitum*, except during exposure
Environmental conditions:	**Temperature:** 21.2-22.3EC **Humidity:** 43.6-51.3% **Air changes:** At least 10/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	17 days (males) or 18 days (females)

B. STUDY DESIGN

1. In life dates: Start: July 24, 2009 End: August 20, 2009

2. Animal assignment: During the pre-test period, animals were acclimated to restraint in nose-only exposure restraint tubes by increasing the restraint time over the acclimation period (one hour on the first day, 2 hours on the second day, 3 hours on the third day, four hours on the fourth day, and six hours on the fifth day). Two days (for males) or three days (for females) prior to the initiation of exposures, animals deemed acceptable for inclusion in the study (based on appropriate food consumption and body weight gain, acclimation to the nose-only restraint system, and lack of physical/clinical abnormalities) were randomly assigned,

stratified by body weight, to the test groups noted in Table 1. Individual body weights at randomization were within ± 20% of the mean body weight for each sex.

TABLE 1: Study design [a]

Test group	Target conc. (mg/m^3)	Analytical conc. (mg/m^3)	Analytical conc. (mg/L) [b]	MMAD Φm	GSD	Rats/sex
Control	0	0	0	0	0	15
Low (LCT)	0.5	0.6/0.5 [c]	0.0006/0.0005	1.9	1.89	15
Mid (MCT)	1.5	1.5	0.0015	1.7	2.03	15
High (HCT)	5.0	4.9	0.0049	1.8	1.79	15

[a] Data were obtained from page 17 and Text Tables 1, 2, and 3 on pages 37 and 38 of the study report.
[b] Analytical concentrations were converted from mg/m^3 to mg/L by the reviewers by dividing by 1000.
[c] Analytical exposures in males/females, respectively

3. **Concentration selection rationale:** It was stated that the exposure concentrations were selected based upon known toxicity information, including the results of a 5-day range-finding study (WIL-544007; Kirkpatrick, Draft). No further information was provided.

4. **Test material administration:** The test substance was administered as a dust aerosol via nose-only inhalation for 6 hours per day, 5 days per week for up to 4 consecutive weeks (up to 20 exposure days).

5. **Generation of the test atmosphere / chamber description:** A diagram of the test atmosphere generation system and exposure chamber was included as Figure 1 on page 602 of the study report. This figure is included in the Appendix in this DER.

   Exposures were conducted using an 11.0-L or 14.1-L conventional nose-only exposure system (designed and fabricated by WIL Research Laboratories, LLC) with synthetic rubber grommets in exposure ports to engage animal holding tubes. One exposure system was dedicated for each group for the duration of the study. Air supplied to the nose-only system was provided from a dry compressed air source. All test atmosphere exhaust passed through the facility exhaust system, which consisted of charcoal- and HEPA-filtration. Exposure chamber temperature, relative humidity, and chamber ventilation rate were continually monitored and manually recorded at approximately 60-minute intervals during the exposure. The mean temperature and mean relative humidity were set for 19-25°C and 30-70%, respectively. All exposure systems were operated under dynamic conditions, with at least 12 air changes per hour, at a slight negative pressure. Oxygen content for the test substance chambers was measured during the method development phase of the study and was at least 19%.

   A dust aerosol atmosphere of the test substance was generated using a Wright Dust Feeder (WDF) and controller, which delivered the test substance aerosol at a constant rate to a stainless steel distribution drum. Dry compressed air was supplied to the WDF. The aerosol from the distribution drum was directed to each test substance exposure system using a

transvector jet. Control animals were exposed to filtered air using an exposure regimen equivalent to the test substance exposures.

Test atmosphere concentrations – Actual exposure concentrations were determined using standard gravimetric methods. Samples were collected on pre-weighed, 25-mm glass-fiber filters held in open-faced filter holders positioned in the animal breathing zones of the nose-only exposure systems. Following sample collection, the filter was re-weighed and the concentration calculated as the filter weight difference divided by the sample volume. One sample was collected weekly for the control exposure system and 2, 3, and 6 samples were collected per exposure day for exposure systems 2, 3, and 4, respectively.

Particle size determination – Aerosol particle size determinations were conducted for each test substance exposure system using a 7-stage stainless steel cascade impactor. At least one sample was collected weekly for each test substance exposure system. For each test substance exposure system, one supplemental particle size determination was performed using an Anderson cascade impactor.

6. **Statistics:** Body weight, body weight change, food consumption, bronchoalveolar lavage fluid, total protein, lactate dehydrogenase, and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant ($p \leq 0.05$) differences among groups, Dunnett's test was used to compare each treated group with the controls. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%. Statistical analyses were not conducted if the number of animals was 2 or less. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. **Cageside observations:** All animals were observed for mortality and moribundity twice daily (once in the morning and once in the afternoon).

b. **Clinical examinations:** Clinical examinations were performed prior to exposure, 0 to 1 hour following exposure (designated as 1 hour post-exposure for report presentation purposes), and once daily on non-exposure days. The absence or presence of findings was recorded for individual animals at the scheduled intervals. Detailed physical examinations were conducted on all animals at least once during the pretest period, at the time of randomization and group assignment, and weekly during the exposure phase (including prior to the scheduled necropsies). On days when detailed physical examinations were conducted, clinical observations were only performed at the post-exposure time point.

2. **Body weight:** Individual body weights were recorded approximately weekly during the pretest period, at the time of randomization and group assignment, and prior to the first exposure. During the exposure period, individual body weights were recorded prior to exposure on Days 4, 11, 18, and 25. Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded on the day of the scheduled necropsies.

3. **Food consumption:** Individual food consumption was recorded approximately weekly during the pretest period and throughout the study. Mean food consumption was calculated as g/animal/day for the corresponding body weight intervals. When food consumption could not be measured for a given interval (due to spillage, weighing error, obvious erroneous value, etc.), the appropriate interval was footnoted as "NA" (Not Applicable) in the summary tables.

4. **Ophthalmoscopic examination:** Ocular examinations were not conducted.

5. **Hematology and clinical chemistry:** Not conducted.

6. **Urinalysis:** Not conducted.

7. **Bronchoalveolar lavage fluid (BALF) evaluation:** During the scheduled necropsy evaluations after 5, 12, or 26 days of exposure, 5 animals/sex/concentration were anesthetized using isoflurane inhalation and euthanized by exsanguination. As soon as possible after exsanguination, the lungs and trachea were removed, weighed, and a ligature was placed on the left mainstem bronchus. BALF samples were obtained via lavage of the right lung, and the following CHECKED (X) parameters were evaluated:

X	Total cell count for alveolar macrophages [a]
X	Differential cell count for alveolar macrophages [b]
X	Neutrophils
X	Lymphocytes
X	Eosinophils
X	Basophils
X	Epithelial cells
X	Lactate dehydrogenase (LDH) [c]
X	Total protein [c]

[a] Performed by WIL Research Laboratories, LLC using a hemocytometer

[b] Performed by Gail L. Walter, MT(ASCP), DVM, DACVP, DABT, using stained Cytospin slides prepared by WIL Research Laboratories, LLC

[c] Performed at WIL Research Laboratories, LLC using a Hitachi 912 Chemistry Analyzer

8. **Pathology:** After completion of the BALF evaluation, the clamp was removed from the left mainstem bronchus, and the lungs were fixed by constant pressure inflation with fixative. A complete necropsy was conducted for all animals. The following CHECKED (X) tissues were collected, fixed in 10% neutral-buffered formalin (except as noted), processed routinely, and stained with hematoxylin and eosin. Following collection of the appropriate protocol-specified tissues, the entire head was removed and preserved. After decalcification, six cross-sections of the nasal cavities were prepared for microscopic examination in accordance to the methods described by Morgan (1991). Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*±
X	Salivary glands*	XX	Heart*±	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*±	X	Eyes (optic nerve)* [b]
X	Jejunum*	XX	Thymus*±		GLANDULAR
X	Ileum*			XX	Adrenal gland*±
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*±	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*±	XX	Testes*± [a]		OTHER
	Gall bladder* (not rat)	XX	Epididymides*± [a]	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin
	RESPIRATORY	XX	Ovaries (and oviducts)*±	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*±	X	Harderian gland
XX	Lungs*	X	Mammary gland (females)* [c]	X	Peyer's patches
X	Nasal cavities* [d]	XX	Cervix		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465

± Organ weights required

[a] Fixed in Bouin's solution

[b] Fixed in Davidson's solution

[c] A corresponding section of skin was collected from the same anatomic location for males.

[d] After decalcification, six cross-sections of the nasal cavities were prepared for microscopic examination in accordance to the methods described by Morgan (1991).

Microscopic examinations were performed on the lungs, liver, kidneys, brain, stomach, skeletal muscle, and gross lesions of all animals (including decedents). A formal pathology peer review was conducted on tissues from the brain, lungs, skeletal muscle, and gross lesions from all animals.

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** Test substance-related mortality was observed in the 0.005 mg/L group females. Two females (nos. 6047 and 6062) were found dead on Day 12, and one female (no. 6029) was found dead on Day 19. Test substance-related clinical observations noted for the animals found dead included the following: hypoactivity, thin body condition, and body cool to touch. Additionally, female no. 6029 was noted with decreased defecation on Day 17, as well as dermal atonia and impaired muscle coordination on Day 18. The majority of these clinical observations were noted within 24 hours of death. The cause of death for these three females was undetermined. These animals had minimal to mild broncho-interstitial pneumonitis characterized by subacute inflammation and an increase in alveolar macrophages. The

characteristics and severities of the microscopic findings in the lungs were similar to those seen in the animals examined at the scheduled necropsies. Two of the three rats that were found dead had mild atrophy, and one of the three animals had minimal degeneration of skeletal muscle (rectus femoris); these skeletal muscle findings were similar in nature and severity to that which occurred in females at the scheduled necropsy on Day 26. From each animal's highest recorded body weight to the last body weight recoded prior to being found dead, animal nos. 6047, 6062, and 6029 had lost 79, 65, and 72 grams, respectively. Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26. All other animals survived to the scheduled necropsies.

2. **Clinical signs of toxicity:** Test substance-related clinical observations were noted for the 0.005 mg/L group females surviving to scheduled termination. These findings included the following: thin body condition noted by Day 11 and continuing throughout the duration of the study, dermal atonia noted from Days 12 to 19, and pale extremities noted in a single animal (female no. 6020) on Day 20. Impaired use of right and left hindlimbs was noted for two animals (female nos. 6020 and 6035) ranging from Day 20 to 24. Other treatment-related clinical findings in this group were limited to yellow and/or red material on various parts of the body (including ocular, nasal, urogenital, and anal). All other clinical findings in the test substance-treated groups were noted with similar incidence in the control group, were limited to single animals, and/or were not noted in a dose-related manner.

B. **BODY WEIGHT AND WEIGHT GAIN:** Selected body weight and body weight gain data are presented in Table 2. Treatment-related effects were observed in both sexes at 0.005 mg/L.

In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased by 8% on Day 4 compared to controls and continued to be decreased by 6-8% (not significant [NS]) throughout the remainder of the study. A significant ($p \leq 0.01$) body weight loss was noted for Days 0-4 in the 0.005 mg/L males (-10 g) compared to a gain of 11 g in the controls, and cumulative body weight gains were 42-56% lower than controls for all other intervals throughout the study; these decreases were statistically significant for all intervals, except for Days 0-25.

In the 0.005 mg/L females, body weights were significantly ($p \leq 0.01$) decreased by 7-36% throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 90% lower ($p \leq 0.01$) than controls for Days 0-4, and cumulative body weight losses of 22-48 g were noted for each of the remaining intervals throughout the study in this group compared to body weight gains of 23-48 g in the control group. There were no other effects of treatment on body weights or body weight gains. Any other significant differences ($p \leq 0.05$) from controls were unrelated to dose.

TABLE 2. Mean (∀SD) body weights and body weight gains (g) during 26 days of treatment with Copper Omadine via inhalation [a]

Day(s)	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Males				
0	274±14.8	275±17.0	272±14.3	274±18.0
4	286±16.2	288±17.8	281±17.9	264±18.1** (↓8)
11	311±15.3	327±27.1	311±26.6	294±17.0 (↓6)
15	326±15.5	356±28.0	330±26.1	304±13.7 (↓7)
18	325±16.6	362±30.4	325±24.3	300±12.6 (↓8)
25	349±18.8	398±33.5* (↑14)	367±32.1	320±22.4 (↓8)
0-4	11±5.8	13±5.9	10±6.9	-10±7.6**
0-18	54±17.6	84±16.8*	50±20.1	24±9.4* (↓56)
0-25	78±21.4	120±20.2*	91±26.2	45±16.6 (↓42)
Females				
0	205±12.7	201±11.3	202±12.0	200±11.1
4	215±12.0	210±12.9	209±13.0	201±12.5** (↓7)
11	231±18.7	226±13.0	224±10.9	181±32.2** (↓22)
14	244±25.4	242±14.5	239±15.1	184±11.8** (↓25)
18	248±27.7	245±14.7	241±16.9	159±9.9** (↓36)
25	257±25.5	255±16.6	250±11.3	180±10.5** (↓30)
0-4	10±3.9	9±4.0	7±4.2	1±6.7**
4-11	14±8.0	14±3.8	12±8.0	-22±31.0**
14-18	4±2.7	3±2.9	2±3.4	-26±21.7**
0-11	23±7.9	23±6.6	19±8.6	-22±32.9**
0-18	39±10.1	37±7.8	33±10.7	-48±11.6**
0-25	48±8.2	47±8.8	42±6.6	-25±3.5**

[a] Data obtained from Tables 11 through 16 on pages 83-93 in the study report. n = 15 for Day 0 and 4, n = 10 for Day 11, and n = 5 for Days 14 onward (except for the 0.005 mg/L females, where n = 4 for Days 14 and 18 and n = 3 for Day 25). Percent differences from the controls are included in parentheses.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

C. FOOD CONSUMPTION

1. **Food consumption:** Food consumption data are presented in Table 3. Food consumption was significantly decreased ($p \leq 0.01$) at 0.005 mg/L for Days 0-4 in the males (↓35%) and for Days 0-4, 4-11, and 11-18 in the females (↓26-40%). Aside from a decrease of 11% ($p \leq 0.05$) in the 0.0015 mg/L females for Days 0-4, food consumption at 0.0005 and 0.0015 mg/mL was comparable to controls throughout the study.

TABLE 3. Mean (∀SD) food consumption (g/animal/day) during 26 days of treatment with Copper Omadine via inhalation[a]

Day(s)	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Males				
-8 to -2	24±1.9	24±1.7	24±1.7	24±1.8
N	15	15	14	15
0 to 4	23±2.1	24±1.9	21±1.8	15±2.4** (↓35)
N	15	15	15	15
4 to 11	23±1.7	25±2.4	22±2.1	21±1.8
N	10	10	10	10
11 to 18	23±0.9	28±2.8* (↑22)	24±2.4	22±1.8
N	5	5	5	5
18 to 25	23±1.9	23±4.8	24±2.3	21±1.3
N	5	5	5	5
Females				
-9 to -3	18±2.4	18±1.8	18±2.5	18±1.7
N	15	15	15	15
0 to 4	19±0.9	18±1.4	17±1.6* (↓11)	13±1.6** (↓32)
N	15	14	15	15
4 to 11	19±1.3	19±1.1	18±1.3	14±4.3** (↓26)
N	10	10	9	10
11 to 18	20±1.7	21±1.1	20±1.9	12±3.0** (↓40)
N	4	5	5	4
18 to 25	20±1.5	20±0.9	20±1.3	20±0.7
N	5	5	5	2

[a] Data were obtained from Tables 17 and 18 on pages 94 and 95 of the study report. Percent differences from the controls are included in parentheses.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

D. SACRIFICE AND PATHOLOGY

1. **Bronchoalveolar lavage fluid (BALF):** Treatment-related effects on BALF samples were found at 0.0015 and 0.005 mg/L in the males (Table 4) and females (Table 5). Lactate dehydrogenase was increased over controls at 0.0015 mg/L in the males on Days 12 and 26 (↑50-59%) and in the females on Day 5 (↑153) and at 0.005 mg/L in the males throughout the study (↑62-161%) and in the females on Days 5 and 12 (↑439-127%). Total protein levels were increased in the 0.0015 and 0.005 mg/L males throughout the study (↑16-138%) and in the 0.0015 and 0.005 mg/L females on Days 5 and 12 (↑66-463%). Total cell counts were increased at 0.0015 and 0.005 mg/L in the males throughout the study (↑42-163%) and in the

females on Day 5 (↑80-278%). Alveolor macrophages were increased in the 0.0015 and 0.005 mg/L males throughout the study (↑26-96%) and in the 0.005 mg/L females on Day 5 (↑193%). With the exception of the 0.005 mg/L females on Day 26, the number of neutrophils was increased throughout the study in the 0.0015 and 0.005 males and females (↑310-12,950%). The number of lymphocytes was increased in the 0.0015 and 0.005 mg/L males throughout the study (↑32-1780%) and in the 0.0015 and 0.005 mg/L females on Day 5 (↑125-986%). The number of eosinophil varied from 0.00 to 0.99 (x 10^6) among the different groups, but did not show a definitive pattern with concentration, time, or sex.

TABLE 4. Mean (±SD) bronchiolar lavage fluid (BALF) absolute values in males during 26 days of treatment with Copper Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Lactate dehydrogenase (U/L)				
Day 5	179±127.06	142.1±64.02	160.3±49.85	290.4±102.29 (↑62)
Day 12	113.6±65.23	97.3±43.17	170.3±54.27 (↑50)	192.6±57.97 (↑70)
Day 26	70.5±14.12	108.1±65.41 (↑53)	112.1±33.16 (↑59)	183.7±160.6* (↑161)
Total protein (mg/dL)				
Day 5	18.4±16.03	18.9±5.50	26.4±6.22 (↑44)	41.5±12.05* (↑126)
Day 12	15.0±5.01	18.0±2.23 (↑20)	26.4±5.00** (↑76)	26.2±4.19** (↑75)
Day 26	11.9±4.83	18.4±9.36 (↑55)	24.0±3.64* (↑102)	28.3±6.96** (↑138)
Total cell count (x 10^6)				
Day 5	10.35±4.029	9.55±5.473	16.06±1.880 (↑55)	21.30±4.628** (↑106)
Day 12	8.14±5.258	9.29±5.408	12.90±4.223 (↑59)	21.40±12.457 (↑163)
Day 26	11.39±5.379	11.55±4.187	16.22±7.073 (↑42)	20.74±6.738 (↑82)
Alveolar macrophages (x 10^6)				
Day 5	10.16±4.147	9.41±5.408	14.79±1.317 (↑46)	15.19±4.161 (↑50)
Day 12	7.87±5.239	8.95±5.215	11.75±4.569 (↑49)	15.41±10.412 (↑96)
Day 26	10.65±5.057	10.34±3.950	13.37±6.382 (↑26)	15.35±8.362 (↑44)
Neutrophils (x 10^6)				
Day 5	0.07±0.048	0.03±0.049	0.66±0.722 (↑843)	5.17±1.347** (↑7286)
Day 12	0.04±0.061	0.05±0.041	0.61±0.615 (↑1425)	5.22±2.711** (↑12,950)
Day 26	0.11±0.107	0.07±0.035	1.57±0.713 (↑1327)	4.01±2.337** (↑3546)
Lymphocytes (x 10^6)				
Day 5	0.05±0.091	0.08±0.105	0.22±0.065 (↑340)	0.94±0.637** (↑1780)
Day 12	0.09±0.038	0.08±0.068	0.22±0.175 (↑144)	0.64±0.507* (↑611)
Day 26	0.41±0.249	0.48±0.665	0.76±0.569 (↑85)	0.54±0.352 (↑32)
Eosinophils (x 10^6)				
Day 5	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
Day 12	0.00±0.000	0.00±0.000	0.00±0.000	0.11±0.185
Day 26	0.00±0.000	0.00±0.000	0.06±0.134	0.08±0.078

[a] Data were obtained from Table 19 on pages 96-102 in the study report. n = 5 for each time point. Percent differences from the controls are included in parentheses.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

TABLE 5. Mean (∀SD) bronchiolar lavage fluid (BALF) absolute values in females during 26 days of treatment with Copper Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Lactate dehydrogenase (U/L)				
Day 5	77.1±14.11	75.9±10.33	195.2±137.64 (↑153)	415.3±274.39** (↑439)
Day 12	145.2±66.81	96.1±39.36	138.8±59.39	329.0±159.83* (↑127)
Day 26	147.4±64.37	114.2±32.53	130.0±52.63	126.1±54.60
Total protein (mg/dL)				
Day 5	8.6±2.05	14.7±3.98 (↑71)	25.6±8.60* (↑198)	48.4±17.73** (↑463)
Day 12	16.5±5.09	19.7±4.75	27.3±6.83* (↑66)	35.9±3.99** (↑118)
Day 26	23.7±15.56	23.6±4.89	29.6±4.13	15.4±4.90
Total cell count (x 10^6)				
Day 5	5.95±2.989	6.74±3.993	10.70±5.824 (↑80)	22.48±9.100** (↑278)
Day 12	8.80±5.704	7.68±1.525	12.23±3.816	11.33±6.998
Day 26	9.34±5.471	6.85±2.414	10.34±4.959	9.14±6.002
Alveolar macrophages (x 10^6)				
Day 5	5.69±2.900	6.60±4.012	9.54±5.303	16.68±7.026** (↑193)
Day 12	8.54±5.850	7.50±1.516	11.08±3.446	8.92±6.350
Day 26	7.77±2.909	6.65±2.341	9.23±4.807	8.90±6.053
Neutrophils (x 10^6)				
Day 5	0.10±0.102	0.01±0.022	0.78±0.368 (↑680)	4.85±1.927** (↑4750)
Day 12	0.04±0.048	0.03±0.038	0.54±0.316 (↑1250)	2.04±1.367** (↑5000)
Day 26	0.20±0.263	0.05±0.049	0.82±0.510* (↑310)	0.00±0.000
Lymphocytes (x 10^6)				
Day 5	0.08±0.062	0.09±0.142	0.18±0.188 (↑125)	0.87±0.493** (↑986)
Day 12	0.09±0.055	0.12±0.039	0.25±0.214	0.26±0.148
Day 26	0.27±0.235	0.08±0.047	0.19±0.151	0.11±0.185
Eosinophils (x 10^6)				
Day 5	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
Day 12	0.00±0.000	0.00±0.000	0.00±0.000	0.04±0.044*
Day 26	0.99±2.165	0.00±0.000	0.01±0.022	0.00±0.000

[a] Data were obtained from Table 20 on pages 107-113 in the study report. $n = 5$ for each time point. Percent differences from the controls are included in parentheses.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

Data for the BALF leukocyte differential parameters are included in Table 6 (males) and Table 7 (females). The proportion of neutrophils increased with concentration and time in both sexes at 0.0015 mg/L (↑329-1122%) and 0.005 mg/L (↑948-4860%), with the exception of the 0.005 mg/L females on Day 26. The remaining three females exposed to 0.005 mg/L had apparently recovered following a dosing-free period (final exposure was administered on Day 18), and neutrophils were not present in the BALF on Day 26. The proportion of lymphocytes was higher than controls in the 0.005 mg/L males and females on Days 5 and 12 (↑136-450%). Due to these increases in neutrophils and lymphocytes, the proportion of alveolar macrophages was lower in the 0.005 mg/L males on Days 5, 12, and 26 (↓24-27%) and females on Days 5 and 12 (↓22-23%). The proportion of alveolar macrophages was minimally to slightly lower than controls in the 0.0015 males and females at all three intervals (↓4-13%). The slightly higher percent of eosinophils in the 0.005 mg/L females on Day 12 was not considered treatment-related (0.3% treated vs 0.0% controls).

TABLE 6. Mean (±SD) bronchiolar lavage fluid (BALF) percent values in males during 26 days of treatment with Copper Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005
Neutrophils (%)				
Day 5	0.6±0.42	0.6±0.89	3.9±3.78 (↑550)	24.7±5.57**(↑4017)
Day 12	0.5±0.35	0.6±0.42	4.7±3.96 (↑840)	24.8±6.81** (↑4860)
Day 26	0.9±1.02	0.6±0.22	11.0±4.72 (↑1122)	21.9±12.24** (↑2333)
Lymphocytes (%)				
Day 5	0.8±1.52	0.8±0.67	1.4±0.55	4.4±2.58** (↑450)
Day 12	1.4±0.96	1.5±2.26	2.1±2.07	3.3±2.51 (↑136)
Day 26	3.3±1.15	4.3±5.72	4.3±2.73	2.9±1.71
Alveolar macrophages (%)				
Day 5	97.4±3.60	98.4±1.56	92.6±7.87 (↓5)	70.9±7.89** (↓27)
Day 12	94.8±6.02	96.0±2.29	89.4±8.04 (↓6)	71.1±8.62** (↓25)
Day 26	93.4±0.65	90.0±11.65	81.3±5.51 (↓13)	70.8±15.55** (↓24)
Eosinophils (%)				
Day 5	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Day 12	0.0±0.00	0.0±0.00	0.0±0.00	0.7±1.04
Day 26	0.0±0.00	0.0±0.00	0.4±0.89	0.5±0.50

[a] Data were obtained from Table 19 on pages 103-106 in the study report. n = 5 for each time point. Percent differences from the controls are included in parentheses.

** Statistically different from the control group at $p \leq 0.01$

TABLE 7. Mean (±SD) bronchiolar lavage fluid (BALF) percent values in females during 26 days of treatment with Copper Omadine via inhalation [a]

Parameter/Interval	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Neutrophils (%)				
Day 5	2.1±1.88	0.1±0.22	9.0±5.49* (↑329)	22.0±3.20** (↑948)
Day 12	0.7±0.84	0.4±0.55	4.8±3.17 (↑586)	21.6±10.63** (↑2986)
Day 26	1.7±1.15	0.6±0.65	11.9±12.19 (↑600)	0.0±0.00
Lymphocytes (%)				
Day 5	1.5±1.17	1.6±2.48	1.4±0.74	3.8±1.52
Day 12	1.6±1.64	1.6±0.55	1.8±1.35	2.9±1.49
Day 26	2.7±1.04	1.5±1.27	2.1±1.34	1.0±1.73
Alveolar macrophages (%)				
Day 5	95.1±1.98	97.1±2.92	88.3±5.16* (↓7)	73.7±3.42** (↓23)
Day 12	94.8±5.61	97.7±0.76	90.9±4.08 (↓4)	74.0±12.60** (↓22)
Day 26	88.8±12.02	97.1±1.08	84.4±14.73 (↓5)	95.2±5.01
Eosinophils (%)				
Day 5	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Day 12	0.0±0.00	0.0±0.00	0.0±0.00	0.3±0.29*
Day 26	5.3±11.30	0.0±0.00	0.2±0.27	0.0±0.00

[a] Data were obtained from Table 20 on pages 114-117 in the study report. n = 5 for each time point. Percent differences from the controls are included in parentheses.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

2. **Organ weight:** Lung weight data are presented in Table 8. At 0.0015 mg/L, relative (to body weight) lung weights were increased by 17% ($p \leq 0.01$) over controls in the males on Day 12. The following increases ($p \leq 0.05$) in lung weights were observed in the 0.005 mg/L animals: (i) relative to body weight (↑25%) and relative to brain weight (↑14%) in the males at Day 5; (ii) absolute (↑18%) and relative to body weight (↑26%) in the males at Day 12; (iii) absolute (↑9%) and relative to body weight (↑20%) in the males at Day 26; (iv) relative to body weight (↑26%) and relative to brain weight (↑19%) in the females at Day 5; (v) relative to body weight in the females at Day 12 (↑35%) and Day 26 (↑48%). Terminal body weights were decreased ($p \leq 0.05$) at this concentration in the males on Day 5 (↓14%) and in the females on Days 12 (↓14%) and 26 (↓33%). There were no treatment-related changes in lung weights at 0.0005 mg/L; the increases ($p \leq 0.05$) in terminal body weight and absolute lung weights in the males at this concentration on Day 26 were unrelated to concentration.

TABLE 8. Mean (±SD) absolute (g) and relative (%) lung weights following inhalation of Copper Omadine [a]

Time interval/Parameter	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Males				
Day 5				
Terminal body weight (g)	264±22.6	260±12.4	260±7.0	227±22.1* (↓14)
Lungs – Absolute (g)	1.21±0.105	1.17±0.102	1.22±0.071	1.30±0.102
Relative to body wt (%)	0.458±0.0250	0.448±0.0240	0.471±0.0217	0.573±0.0352** (↑25)
Relative to brain wt (%)	62.070±5.1369	60.138±5.4791	63.287±2.6926	70.828±5.3190* (↑14)
Day 12				
Terminal body weight (g)	280±14.1	280±21.8	271±28.8	262±16.5
Lungs – Absolute (g)	1.16±0.052	1.19±0.121	1.32±0.178	1.37±0.100* (↑18)
Relative to body wt (%)	0.417±0.0183	0.424±0.0244	0.487±0.0353** (↑17)	0.524±0.406** (↑26)
Relative to brain wt (%)	61.661±4.1627	61.161±4.8121	68.517±8.8796	69.969±7.4820
Day 26				
Terminal body weight (g)	314±18.6	363±33.6* (↑16)	328±27.5	285±19.8
Lungs – Absolute (g)	1.29±0.022	1.41±0.093* (↑9)	1.38±0.050	1.40±0.057* (↑9)
Relative to body wt (%)	0.410±0.0236	0.389±0.0270	0.423±0.0217	0.491±0.0277** (↑20)
Relative to brain wt (%)	65.769±4.4898	70.621±7.1210	68.665±2.7699	71.576±5.4007
Females				
Day 5				
Terminal body weight (g)	182±6.6	180±13.4	181±10.1	169±9.5
Lungs – Absolute (g)	0.95±0.078	0.96±0.110	1.00±0.040	1.11±0.137
Relative to body wt (%)	0.523±0.0423	0.532±0.0306	0.554±0.0291	0.657±0.0563** (↑26)
Relative to brain wt (%)	50.889±4.4019	52.870±5.5596	53.776±3.2620	60.786±6.1344* (↑19)
Day 12				
Terminal body weight (g)	200±8.5	193±10.5	199±13.0	172±23.7* (↓14)
Lungs – Absolute (g)	1.01±0.139	1.03±0.184	1.15±0.106	1.17±0.184
Relative to body wt (%)	0.502±0.0532	0.536±0.0898	0.581±0.0448	0.678±0.0420** (↑35)
Relative to brain wt (%)	52.706±3.5169	55.476±7.5187	62.696±8.0474	63.436±8.1892
Day 26				
Terminal body weight (g)	231±27.2	227±15.3	224±14.1	154±12.7** (↓33)
Lungs – Absolute (g)	1.15±0.125	1.13±0.108	1.16±0.095	1.14±0.125
Relative to body wt (%)	0.501±0.0618	0.498±0.0522	0.516±0.0337	0.741±0.0597** (↑48)
Relative to brain wt (%)	60.725±7.7735	59.871±5.9303	60.583±4.4969	61.934±5.1543

[a] Data obtained from Tables 28 through 33 on pages 125-169 of the study report. Percent differences from controls are included in parentheses. n = 5 in all groups except for the 0.005 mg/L females where n = 5 on Day 5, n = 4 on Day 12, and n = 3 on Day 26.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

Thymus weight data are presented in Table 9. The following decreases ($p \leq 0.05$) in thymus weights were observed in the 0.005 mg/L animals: (i) absolute (↓36%) and relative to brain weight (↓32%) in the males at Day 5; (ii) absolute (↓34%), relative to body weight (↓26%), and relative to brain weight (↓33%) in males on Day 26; and (iii) absolute (↓41%), relative to body weight (↓37%), and relative to brain weight (↓40%) in females on Day 5. Additionally at this concentration, terminal body weights were decreased by 14% ($p \leq 0.05$) in the males on Day 5, by 14% ($p \leq 0.05$) in the females on Day 12, and by 33% in the females on Day 26. Non-significant (NS) decreases in thymus weights were observed in the 0.005 mg/L females on Day 12 (↓13-25%) and on Day 26 (↓20-22%) with the exception of the increase of 14% in relative to body weight).

TABLE 9. Mean (∀ SD) absolute (g) and relative (%) thymus weights following inhalation of Copper Omadine [a]

Time interval/Parameter	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Males				
Day 5				
Terminal body weight (g)	264±22.6	260±12.4	260±7.0	227±22.1* (↓14)
Thymus – Absolute (g)	0.4870±0.02744	0.4973±0.08451	0.4453±0.11796	0.3109±0.05931** (↓36)
Relative to body wt (%)	0.185±0.0168	0.192±0.0346	0.712±0.0461	0.138±0.0293 (↓25)
Relative to brain wt (%)	25.034±1.4970	25.553±4.0332	23.081±6.0942	16.988±3.6318* (↓32)
Day 12				
Terminal body weight (g)	280±14.1	280±21.8	271±28.8	262±16.5 (↓6)
Thymus – Absolute (g)	0.4988±0.15004	0.4784±0.07977	0.4448±0.16079	0.4417±0.11904 (↓11)
Relative to body wt (%)	0.177±0.0456	0.171±0.0209	0.161±0.0463	0.167±0.0380 (↓6)
Relative to brain wt (%)	26.231±6.8621	24.676±4.0605	23.154±8.5200	22.318±4.8684 (↓15)
Day 26				
Terminal body weight (g)	314±18.6	363±33.6* (↑16)	328±27.5	285±19.8 (↓9)
Thymus – Absolute (g)	0.4157±0.09854	0.5056±0.07097	0.4264±0.08369	0.2749±0.05084* (↓34)
Relative to body wt (%)	0.131±0.0260	0.140±0.0190	0.130±0.0219	0.097±0.0169* (↓26)
Relative to brain wt (%)	21.084±4.2146	25.281±2.9743	21.117±3.7460	14.075±2.6909* (↓33)
Females				
Day 5				
Terminal body weight (g)	182±6.6	180±13.4	181±10.1	169±9.5 (↓7)
Thymus – Absolute (g)	0.4298±0.08417	0.4033±0.09870	0.3943±0.06253	0.2525±0.06617**
Relative to body wt (%)	0.235±0.0425	0.224±0.0537	0.219±0.0394	0.149±0.0385* (↓37)
Relative to brain wt (%)	22.969±4.7755	22.116±4.7251	21.205±3.5523	13.776±3.3165** (↓40)
Day 12				
Terminal body weight (g)	200±8.5	193±10.5	199±13.0	172±23.7* (↓14)
Thymus – Absolute (g)	0.3995±0.08975	0.4432±0.09049	0.4254±0.06242	0.3009±0.07349 (↓25)
Relative to body wt (%)	0.199±0.0407	0.231±0.0539	0.216±0.0439	0.174±0.0213 (↓13)
Relative to brain wt (%)	21.226±5.6772	23.911±4.7429	22.984±2.7653	16.254±2.9420 (↓23)
Day 26				
Terminal body weight (g)	231±27.2	227±15.3	224±14.1	154±12.7** (↓33)
Thymus – Absolute (g)	0.4238±0.03099	0.3630±0.06557	0.3430±0.07613	0.3310±0.13815 (↓22)
Relative to body wt (%)	0.184±0.0125	0.161±0.0294	0.153±0.0357	0.210±0.0721 (↑14)
Relative to brain wt (%)	22.328±2.0170	19.325±3.7677	18.013±4.4896	17.819±6.9913 (↓20)

[a] Data obtained from Tables 28 through 33 on pages 125-169 of the study report. Percent differences from controls are included in parentheses. n = 5 in all groups except for the 0.005 mg/L females where n = 5 on Day 5, n = 4 on Day 12, and n = 3 on Day 26.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

Increases ($p \leq 0.05$) in relative to body weight (↑79%) and relative to brain weight (↑24%) adrenal weights were noted in the 0.005 mg/L females on Day 26 compared to controls, accompanied by a decrease of 33% ($p \leq 0.01$) in terminal body weights (Table 10). However, absolute and relative (to body weight and brain weight) adrenal weights were increased by 23-24% over controls ($p \leq 0.05$) in the 0.0005 mg/L females on Day 26, with terminal body weights similar to controls. These increases are considered unrelated to treatment because they are not concentration-dependent. Furthermore, it was stated that these data were within the range of historical controls (data not presented). Finally, it was stated that the increased adrenal weights did not correspond to any microscopic findings, although the adrenal glands were not listed as one of the organs evaluated microscopically and no histopathology data were presented.

There were no other treatment-related effects on organ weights. Several statistically significant ($p \leq 0.05$) differences from controls were noted at 0.005 mg/L but were likely due to the decreased terminal body weights in these animals.

TABLE 10. Mean (∀ SD) absolute (g) and relative (%) adrenal weights in females following inhalation of Copper Omadine [a]

Time interval/Parameter	Analytical concentration (mg/L)			
	0	0.0005	0.0015	0.005 [b]
Day 5				
Terminal body weight (g)	182±6.6	180±13.4	181±10.1	169±9.5
Adrenals – Absolute (g)	0.0667±0.00853	0.0662±0.00486	0.0693±0.00846	0.0719±0.01054
Relative to body wt (%)	0.037±0.0057	0.037±0.0033	0.038±0.0044	0.042±0.0057
Relative to brain wt (%)	3.562±0.5135	3.666±0.3794	3.719±0.4189	3.942±0.5970
Day 12				
Terminal body weight (g)	200±8.5	193±10.5	199±13.0	172±23.7* (↓14)
Adrenals – Absolute (g)	0.0838±0.01771	0.0754±0.01208	0.0727±0.01172	0.0744±0.00350
Relative to body wt (%)	0.042±0.0081	0.039±0.0070	0.037±0.0056	0.044±0.0055
Relative to brain wt (%)	4.387±0.6222	4.073±0.6677	3.945±0.6745	4.067±0.3412
Day 26				
Terminal body weight (g)	231±27.2	227±15.3	224±14.1	154±12.7** (↓33)
Adrenals – Absolute (g)	0.0663±0.00815	0.0814±0.00886* (↑23)	0.0722±0.00262	0.0798±0.00755
Relative to body wt (%)	0.029±0.0038	0.036±0.0063* (↑24)	0.032±0.0027	0.052±0.0015** (↑79)
Relative to brain wt (%)	3.487±0.4440	4.330±0.4789* (↑24)	3.784±0.2589	4.327±0.3117* (↑24)

[a] Data obtained from Tables 29, 31, and 33 on pages 133, 149, and 165 of the study report. Percent differences from controls are included in parentheses. n = 5 in all groups except for the 0.005 mg/L females where n = 5 on Day 5, n = 4 on Day 12, and n = 3 on Day 26.

[b] Due to mortality, the exposures for the 0.005 mg/L group females were terminated following 3 weeks of exposure. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

* Statistically different from the control group at $p \leq 0.05$

** Statistically different from the control group at $p \leq 0.01$

3. **Gross pathology:** Data on the incidences of enlarged lymph nodes are presented in Table 11. Enlarged bronchial lymph nodes were noted in one 0.005 mg/L female on Day 5 (#6033), one 0.0015 mg/L male on Day 12 (#5990), two 0.005 mg/L males on Day 12 (#5962 and 6010), one 0.005 mg/L female on Day 12 (#6081), and one 0.005 mg/L male on Day 26 (#5997). Enlarged mediastinal lymph nodes were noted in one 0.0015 mg/L male on Day 12 (#5990), one 0.005 mg/L male on Day 12 (#6010), two 0.005 mg/L females on Day 12 (#6018 and 6081), and one 0.005 mg/L male on Day 26 (#5997). These findings, noted at gross necropsy, corresponded to lymphoid hyperplasia confirmed upon microscopic examination. There were no other macroscopic findings which could be attributed to treatment.

TABLE 11. Incidence (# affected) of lymph node hyperplasia in animals at scheduled terminations [a]

Study Day/Tissue	Analytical concentration (mg/L)							
	0	0.0005	0.0015	0.005	0	0.0005	0.0015	0.005
	Males				Females			
Day 5								
Bronchial	0	0	0	0	0	0	0	1
Day 12								
Bronchial	0	0	1	2	0	0	0	1
Mediastinal	0	0	1	1	0	0	0	2
Day 26								
Bronchial	0	0	0	1	0	0	0	0
Mediastinal	0	0	0	1	0	0	0	0

[a] Data obtained from Text Tables 4, 5, and 6 on pages 668 and 669 of the study report.

4. **Microscopic pathology:** The remaining treatment-related microscopic findings (aside from the lymphoid hyperplasia detailed above) are included below in Table 12.

In the lungs, subacute inflammation was observed in the males at 0.0015 mg/L (4/15) and 0.005 mg/L (13/15) compared to controls (0/15) and in the females at 0.005 mg/L (9/12) compared to controls (1/15). The severity of subacute inflammation of the lungs ranged from minimal to moderate in the males and from minimal to mild in the females. Minimal alveolar macrophages were observed in the 0.0015 mg/L males (5/15) and females (3/15), and mild alveolar macrophages were found at 0.005 mg/L in the males (12/15) and females (9/12) compared to controls (0/15 males; 1/15 females). Perivasculitis was found in the 0.005 mg/L males (1/15) and females (2/12) compared to controls (0/15 males; 1/15 females). These findings were considered to be due to direct effects of the test material on the respiratory tract. With the exception of the perivasculitis in the females, the findings increased in severity with increasing concentration.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to moderate degeneration (4/15 treated vs 0/15 controls) and mild to moderate atrophy (5/15 treated vs 0/15 controls). There were no other treatment-related findings in the organs and tissues examined microscopically.

TABLE 12. Incidence (# affected) of selected microscopic findings in animals at scheduled terminations [a]

Microscopic finding		Analytical concentration (mg/L)							
		0	0.0005	0.0015	0.005	0	0.0005	0.0015	0.005
		Males				Females			
Lungs (number examined)		15	15	15	15	15	15	15	12
Inflammation, subacute –	Total	0	0	4	13	1	0	0	9
	Minimal	---	---	4	1	0	---	---	1
	Mild	---	---	0	11	1	---	---	8
	Moderate	---	---	0	1	0	---	---	0
Alveolar macrophages –	Total	0	0	5	12	1	0	3	9
	Minimal	---	---	5	0	0	---	3	0
	Mild	---	---	0	12	1	---	0	9
Perivasculitis –	Total	0	0	0	1	1	0	0	2
	Minimal	---	---	---	0	0	---	---	2
	Mild	---	---	---	1	0	---	---	0
	Moderate	---	---	---	0	1	---	---	0
Skeletal muscle (number examined)		15	15	15	15	15	15	15	15
Degeneration –	Total	0	0	0	0	0	0	0	4
	Minimal	---	---	---	---	---	---	---	1
	Mild	---	---	---	---	---	---	---	2
	Moderate	---	---	---	---	---	---	---	1
Atrophy –	Total	0	0	0	0	0	0	0	5
	Mild	---	---	---	---	---	---	---	2
	Moderate	---	---	---	---	---	---	---	3

[a] Data obtained from Text Table 7 on page 670 of the study report.
--- Severity incidence not applicable because total incidence was zero.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the LOAEL was 0.0015 mg/L based on local (port of entry) effects on BALF parameters, lung weights, and microscopic findings in the lungs (broncho-interstitial pneumonitis) and lung-draining lymph nodes. Systemic toxicity was observed at 0.005 mg/L, and was characterized by increased mortality, clinical signs of toxicity, and skeletal muscle degeneration and atrophy in the females and on decreased body weights, body weight gains, and food consumption in both sexes. The NOAEL was 0.0005 mg/L.

B. REVIEWER COMMENTS: Test substance-related mortality was observed in the 0.005 mg/L group females. Two females (nos. 6047 and 6062) were found dead on Day 12, and one female (no. 6029) was found dead on Day 19. Test substance-related clinical observations noted for the animals found dead included hypoactivity, thin body condition, and body cool to touch. Additionally, female no. 6029 was noted with decreased defecation on Day 17, as well as dermal atonia and impaired muscle coordination on Day 18. The majority of these clinical observations were noted within 24 hours of death. However, the cause of death for

these three females was undetermined. Microscopic findings in the lungs (broncho-interstitial pneumonitis characterized by subacute inflammation and an increase in alveolar macrophages) and skeletal muscle (atrophy and degeneration) were similar in nature and severity to that which occurred in females at the scheduled necropsy on Day 26. The three surviving females in this group were held without further exposures between Day 19 and the scheduled necropsy on Day 26.

Test substance-related clinical observations were noted for the 0.005 mg/L group females surviving to scheduled termination. These findings included the following: thin body condition noted by Day 11 and continuing throughout the duration of the study, dermal atonia noted from Days 12 to 19, and pale extremities noted in a single animal on Day 20. Impaired use of right and left hindlimbs was noted for two animals ranging from Day 20 to 24. Other treatment-related clinical findings in this group were limited to yellow and/or red material on various parts of the body (including ocular, nasal, urogenital, and anal).

Treatment-related effects on body weights were observed in both sexes at 0.005 mg/L. In the 0.005 mg/L males, body weights were significantly ($p \leq 0.01$) decreased on Day 4 and continued to be decreased (NS) throughout the remainder of the study. A significant ($p \leq 0.01$) body weight loss was noted for Days 0-4 in the 0.005 mg/L males (-10 g) compared to a gain of 11 g in the controls, and cumulative body weight gains were 42-56% lower than controls for all other intervals throughout the study; these decreases were statistically significant for all intervals, except for Days 0-25. In the 0.005 mg/L females, body weights were significantly ($p \leq 0.01$) decreased throughout the study. Cumulative body weight gains in the 0.005 mg/L females were 90% lower ($p \leq 0.01$) than controls for Days 0-4, and cumulative body weight losses of 22-48 g were noted for each of the remaining intervals throughout the study in this group compared to body weight gains of 23-48 g in the control group.

Food consumption was decreased ($p \leq 0.05$) in the 0.0015 mg/L females for Days 0-4. Additionally at 0.005 mg/L, food consumption was decreased ($p \leq 0.01$) for Days 0-4 in the males and for Days 0-4, 4-11, and 11-18 in the females.

Treatment-related effects on BALF samples were found at 0.0015 and 0.005 mg/L in both sexes. Lactate dehydrogenase was increased over controls at 0.0015 mg/L in the males on Days 12 and 26 and in the females on Day 5 and at 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total protein levels were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Days 5 and 12. Total cell counts and the number of lymphocytes were increased at 0.0015 and 0.005 mg/L in the males throughout the study and in the females on Day 5. Alveolar macrophages were increased in the 0.0015 and 0.005 mg/L males throughout the study and in the 0.005 mg/L females on Day 5. With the exception of the 0.005 mg/L females on Day 26, the number of neutrophils was increased throughout the study in the 0.0015 and 0.005 mg/L males and females.

Examination of the BALF leukocyte differential data showed that the proportion of neutrophils increased with concentration and time in both sexes at 0.0015 and 0.005 mg/L, with the exception of the 0.005 mg/L females on Day 26. The remaining three females

exposed to 0.005 mg/L had apparently recovered (following the final exposure on Day 18), and neutrophils were not present in the BALF on Day 26. The proportion of lymphocytes was higher than controls in the 0.005 mg/L males and females on Days 5 and 12. Due to these increases in neutrophils and lymphocytes, the proportion of alveolar macrophages was lower in the 0.005 mg/L males on Days 5, 12, and 26 and females on Days 5 and 12. The proportion of alveolar macrophages was slightly lower than controls at 0.0015 mg/L in both sexes at all three intervals.

At 0.0015 mg/L, relative (to body weight) lung weights were increased ($p \leq 0.01$) over controls in the males on Day 12. The following increases ($p \leq 0.05$) in lung weights were observed in the 0.005 mg/L animals: (i) relative to body weight and relative to brain weight in the males and females at Day 5; (ii) absolute and relative to body weight in the males at Days 12 and 26; and (iii) relative to body weight in the females at Days 12 and 26. Terminal body weights were decreased ($p \leq 0.05$) at this concentration in the males on Day 5 and in the females on Days 12 and 26. Subacute inflammation was observed in the lungs in males at 0.0015 mg/L (4/15) and 0.005 mg/L (13/15) compared to controls (0/15) and in the females at 0.005 mg/L (9/12) compared to controls (1/15). The severity of subacute inflammation of the lungs ranged from minimal to moderate in the males and from minimal to mild in the females. Minimal alveolar macrophages were observed in the 0.0015 mg/L males (5/15) and females (3/15), and mild alveolar macrophages were found at 0.005 mg/L in the males (12/15) and females (9/12) compared to controls (0/15 males; 1/15 females). Perivasculitis was found in the 0.005 mg/L males (1/15) and females (2/12) compared to controls (0/15 males; 1/15 females). These findings were considered to be due to direct effects of the test material on the respiratory tract. With the exception of the perivasculitis in the females, the findings increased in severity with increasing concentration.

Absolute and relative to brain weight thymus weights were decreased ($p \leq 0.05$) in the males at Day 5. Absolute, relative to body weight, and relative to brain weight thymus weights were decreased ($p \leq 0.05$) in males on Day 26 and in the females on Day 5. Additionally at this concentration, terminal body weights were decreased ($p \leq 0.05$) in the males on Day 5 and in the females on Days 12 and 26. The investigators stated that lower thymus weight is a common stress response in the rat. However, because the thymus was not examined microscopically, these decreases are considered to be of equivocal toxicological significance.

Enlarged bronchial lymph nodes were noted in one 0.005 mg/L female on Day 5, one 0.0015 mg/L male on Day 12, two 0.005 mg/L males on Day 12, one 0.005 mg/L female on Day 12, and one 0.005 mg/L male on Day 26. Enlarged mediastinal lymph nodes were noted in one 0.0015 mg/L male on Day 12, one 0.005 mg/L male on Day 12, two 0.005 mg/L females on Day 12, and one 0.005 mg/L male on Day 26. These findings, noted at gross necropsy, corresponded to lymphoid hyperplasia confirmed upon microscopic examination. The investigators reported that the lymph node findings represent a common reactive or secondary response to inhalation of a dust aerosol and to the microscopic changes observed in the lung.

Examination of the skeletal muscle in the 0.005 mg/L females revealed minimal to moderate degeneration (4/15 treated vs 0/15 controls) and mild to moderate atrophy (5/15 treated vs 0/15 controls).

The LOAEL is 0.0015 mg/L based on decreased food consumption in the females, effects on BALF parameters (increased LDH, total protein, total cell counts, lymphocytes, alveolar macrophages, and neutrophils) and histopathology in the lungs (subacute inflammation and alveolar macrophages) in both sexes. The NOAEL is 0.0005 mg/L.

This 28-day study is classified as **acceptable/non-guideline.** This study was conducted after review of a protocol submitted by the registrant as part of discussions between the Antimicrobials Division and the registrant to determine toxic similarity between zinc pyrithione and copper pyrithione. The purpose of this study with copper pyrithione was to examine toxic effects of copper pyrithione by inhalation after 4 weeks, which included examination of lung bronchioalveolar lavage fluid after single or repeated inhalation exposures, and examination of lung histopathology. Certain parameters (microscopic examination of nasal passages, trachea, and larynx; neurobehavioral, ophthalmologic, and clinical pathology examinations) were not conducted in this study. A similar study has been conducted with zinc pyrithione (MRID 48006404).

C. **STUDY DEFICIENCIES:** The following study deficiencies were found:

- Microscopic examinations were only conducted on the stomach, liver, lungs, kidneys, brain, skeletal muscle, and gross lesions. Therefore, the majority of the required tissues collected for histopathology were not examined microscopically. Given the fact that treatment-related effects were observed in both sexes in the lungs and BALF, the lack of examination of the more anterior tissues of the respiratory system (including the nasal tissues, larynx, and trachea) was considered a major deficiency.

- EPA Guideline OPPTS 870.3465 requires a minimum of 10 rats/sex/concentration; however, only 5 rats/sex/concentration were used in this study for each sacrifice period (i.e., n = 5 for BALF, organ weights, gross pathology, and histopathology endpoints). Although OECD Guideline No. 412 considers 5 rats/sex/concentration an acceptable number of animals, using fewer animals results not only in a loss of statistical power to discern differences but also makes interpretation of the data challenging in general. However, this deficiency would have a greater impact on the study's acceptability if a NOAEL and LOAEL had not been observed.

- Ophthalmoscopic examinations were not performed.

- Hematology and clinical chemistry evaluations were not conducted.

- This study was conducted for 26 days, instead of the 90 days required by Guideline OPPTS 870.3465. However, the EPA routinely accepts inhalation studies that have been conducted for 28 days; therefore, this study was considered to be of sufficient duration.

APPENDIX

FIGURE 1: ATMOSPHERE GENERATION AND EXPOSURE SYSTEM